

Effect of increased milk intake combined with endurance exercise training on body composition, blood-lipids and inflammatory markers in overweight young males

Rebekah Roberts, B.Kin.

Submitted in partial fulfilment of the requirements for the degree of  
Master of Science in Applied Health Sciences (Kinesiology)

Supervisor: Dr. Brian D. Roy

Faculty of Applied Health Sciences  
Brock University,  
St. Catharines, ON

Rebekah Roberts © 2013

**Abstract –**

Consuming low-fat milk (LFM) after resistance training leads to improvements in body composition. Habitual aerobic exercise and dairy intake are relatively easy lifestyle modifications that could benefit a population at risk for becoming obese. Thus, the purpose of this study was to investigate combining increased LFM intake with endurance exercise on body composition, blood-lipid profile and metabolic markers. 40 young males were randomized into four groups: one ingesting 750mL LFM immediately post-exercise, the other 6hrs post-exercise; and two isocaloric carbohydrate groups ingesting at the two different times. Participants completed a 12 week endurance-training program (cycling 1 hour/day at ~60%  $\text{VO}_2\text{peak}$ , 5 days/week). 23 participants completed the study. Increases in lean mass ( $p < 0.05$ ), and decreases in anti-inflammatory marker adiponectin ( $p < 0.05$ ) were seen in all groups. No other significant changes were observed. Future analyses should focus on longer duration exercise and include a larger sample.

## **Acknowledgements**

Thanks to all the individuals and organizations who made this thesis possible through funding, preparation of the methodology and rationale for the study, flexibility in scheduling pre- and post-testing measures, patience through various roadblocks and ‘surprises’ encountered along the way, as well as the many people who gave their input into the writing process.

Special recognition to:

Dr. Mark Tarnopolsky, Erin Hatcher, and Alissa Aboud at McMaster University;

Dr. Brian Roy, Dr. Wendy Ward, Dr. Sandra Peters, and Dr. Paul LeBlanc, as well as Elizabeth Euler, Laura Castelli, and Paula Miotto at the Brock University Centre for Bone and Muscle Health;

Geoff Hartley at the Brock University Environmental Ergonomics Lab;  
and the Dairy Farmers of Canada.

## Table of Contents

|  |             |
|--|-------------|
| <b>Abstract</b>  | <b>ii</b>   |
| <b>Acknowledgements</b>                                  | <b>iii</b>  |
| <b>List of Figures</b>                                   | <b>vi</b>   |
| <b>List of Tables</b>                                    | <b>vii</b>  |
| <b>List of Abbreviations</b>                             | <b>viii</b> |
| <b>1. Introduction</b>                                   |             |
| <b>1.1 Background – What is obesity?</b>                 | <b>1</b>    |
| <b>2. Review of Literature</b>                           |             |
| <b>2.1 Increased body mass</b>                           | <b>2</b>    |
| <b>2.2 Inflammation</b>                                  | <b>3</b>    |
| <b>2.3 Interventions</b>                                 | <b>7</b>    |
| 2.3.1 <i>Bovine milk components and body composition</i> | <b>12</b>   |
| 2.3.2 <i>Bovine milk components and inflammation</i>     | <b>17</b>   |
| 2.3.3 <i>Exercise and body composition</i>               | <b>18</b>   |
| 2.3.4 <i>Exercise and inflammation</i>                   | <b>19</b>   |
| 2.3.5 <i>Bovine milk and exercise in combination</i>     | <b>20</b>   |
| 2.3.6 <i>Timing of nutritional intervention</i>          | <b>23</b>   |
| <b>3. Statement of the problem</b>                       | <b>25</b>   |
| <b>4. Purpose</b>  | <b>27</b>   |
| <b>5. Hypotheses</b>                                     | <b>28</b>   |
| <b>6. Methods</b>  |             |
| <b>6.1 Participant recruitment</b>                       | <b>30</b>   |

|   |    |
|---|----|
| 6.2 Pre-testing measures                                      | 32 |
| 6.3 Training protocol and post-testing measures               | 33 |
| 7. Analyses   | 35 |
| 8. Results  |    |
| 8.1 Baseline  | 37 |
| 8.2 Dietary intake  | 39 |
| 8.3 Endurance exercise capacity markers                       | 44 |
| 8.4 Body composition  | 46 |
| 8.5 Blood lipids  | 48 |
| 8.6 Inflammatory markers                                      | 51 |
| 8.7 Metabolic markers   | 53 |
| 9. Discussion   |    |
| 9.1 Vitamin D – intake and serum levels                       | 55 |
| 9.2 Body composition  | 57 |
| 9.2.1 Calcium/dairy intake, and exercise for body composition | 57 |
| 9.2.2 Lean mass gain  | 59 |
| 9.3 VO <sub>2</sub> peak                                      | 60 |
| 9.4 Blood lipids  | 60 |
| 9.5 Inflammatory and anti-inflammatory markers                | 63 |
| 9.5.1 Dairy intake and inflammation                           | 64 |
| 9.5.2 Exercise and inflammation                               | 64 |
| 9.6 Metabolic markers   | 66 |
| 9.6.1 Fasting insulin   | 66 |

|  |           |
|--|-----------|
| 9.6.2 <i>Fasting glucose and HOMA index</i>              | 67        |
| <b>9.7 Summary of findings</b>                           | <b>69</b> |
| <b>9.8 Implications and strengths</b>                    | <b>70</b> |
| <b>9.9 Limitations and future directions</b>             | <b>71</b> |
| <b>10. Conclusions</b>                                   | <b>74</b> |
| <b>11. References</b>                                    | <b>75</b> |
| <b>12. Appendices</b>                                    |           |
| <b>12.1 Pre- and post-testing data</b>                   | <b>83</b> |
| 12.1.1 <i>Nutritional intake</i>                         | 83        |
| 12.1.2 <i>Serum vitamin D</i>                            | 84        |
| 12.1.3 <i>Body composition</i>                           | 82        |
| 12.1.4 <i>VO<sub>2</sub>peak</i>                         | 86        |
| 12.1.5 <i>Serum lipids</i>                               | 87        |
| 12.1.6 <i>Inflammatory and anti-inflammatory markers</i> | 89        |
| 12.1.7 <i>Metabolic markers</i>                          | 91        |

## List of Figures

|   |           |
|---|-----------|
| <b>Figure 1.</b> Proposed role of adipokine action  | <b>6</b>  |
| <b>Figure 2.</b> Proposed mechanisms through which lifestyle interventions may attenuate obesity-related risk factors | <b>11</b> |
| <b>Figure 3.</b> Outline of experimental protocol   | <b>31</b> |
| <b>Figure 4.</b> Influence of beverage type and intake timing on change in serum-25-(OH)D3                            | <b>43</b> |
| <b>Figure 5.</b> Influence of beverage type and intake timing on change in VO <sub>2</sub> peak measures              | <b>45</b> |
| <b>Figure 6.</b> Influence of beverage type and intake timing on changes in body composition                          | <b>47</b> |
| <b>Figure 7.</b> Influence of beverage type and intake timing on change in total blood cholesterol concentration      | <b>49</b> |
| <b>Figure 8.</b> Influence of beverage type and intake timing on change in inflammatory markers                       | <b>52</b> |
| <b>Figure A1.</b> Vitamin D status by group and time point.   | <b>84</b> |
| <b>Figure A2.</b> Total cholesterol by group and time point.  | <b>87</b> |
| <b>Figure A3.</b> TNF- $\alpha$ (A), CRP (B) and adiponectin (C) by group and time point                              | <b>89</b> |

## List of Tables

|  |           |
|--|-----------|
| <b>Table 1.</b> Summary of studies with dietary measures   | <b>8</b>  |
| <b>Table 2.</b> Summary of studies with exercise measures  | <b>10</b> |
| <b>Table 3.</b> Baseline characteristics   | <b>38</b> |
| <b>Table 4.</b> Total energy and macronutrient intake  | <b>40</b> |
| <b>Table 5.</b> Influence of beverage type and intake timing on change in serum lipids.                    | <b>50</b> |
| <b>Table 6.</b> Influence of beverage type and intake timing on change in fasting serum metabolic markers. | <b>54</b> |
| <b>Table A1.</b> Total energy and macronutrient intake by group and time point                             | <b>83</b> |
| <b>Table A2.</b> Body composition measures by group and time point   | <b>85</b> |
| <b>Table A3.</b> VO <sub>2</sub> peak measures by group and time point                                     | <b>86</b> |
| <b>Table A4.</b> Serum lipids by group and time point  | <b>88</b> |
| <b>Table A5.</b> Fasting inflammatory markers by group and time point                                      | <b>90</b> |
| <b>Table A6.</b> Fasting serum metabolic markers by group and time point                                   | <b>91</b> |



### List of Abbreviations

|                        |                                      |
|------------------------|--------------------------------------|
| <b>°C</b>              | Degrees Celsius                      |
| <b>μU</b>              | Microunit(s)                         |
| <b>1,25-(OH)D</b>      | 1,25-dihydroxyvitamin D / calcitriol |
| <b>25-(OH)D3</b>       | 25-hydroxyvitamin D3                 |
| <b>ACE</b>             | Angiotensin Converting Enzyme        |
| <b>ANOVA</b>           | Analysis Of Variance                 |
| <b>BCAA</b>            | Branched-Chain Amino Acids           |
| <b>BMI</b>             | Body Mass Index                      |
| <b>BW</b>              | Body Weight                          |
| <b>Ca<sup>2+</sup></b> | Calcium                              |
| <b>CD</b>              | Control Diet                         |
| <b>CEA</b>             | Carbohydrate-Exercise-Alternate      |
| <b>CEI</b>             | Carbohydrate-Exercise-Immediate      |
| <b>CHO</b>             | Carbohydrate                         |
| <b>CRP</b>             | C-Reactive Protein                   |
| <b>D</b>               | Day(s)                               |
| <b>DEA</b>             | Dairy-Exercise-Alternate             |
| <b>DEI</b>             | Dairy-Exercise-Immediate             |
| <b>DXA</b>             | Dual X-ray Absorptiometry            |
| <b>ELISA</b>           | Enzyme-linked immunosorbent assay    |
| <b>FM</b>              | Fat Mass                             |
| <b>g</b>               | Gram(s)                              |

|              |                              |
|--------------|------------------------------|
| <b>HC/HP</b> | High Calcium/High Protein    |
| <b>HC/NP</b> | High Calcium/Normal Protein  |
| <b>HD</b>    | High Dairy                   |
| <b>HDL</b>   | High Density Lipoprotein     |
| <b>HOMA</b>  | Homeostatic Model Assessment |
| <b>HR</b>    | Heart Rate                   |
| <b>hr</b>    | Hour                         |
| <b>HRmax</b> | Maximal Heart Rate           |
| <b>IL-6</b>  | Interleukin-6                |
| <b>IU</b>    | International Unit(s)        |
| <b>Kcal</b>  | Kilocalorie(s)               |
| <b>kg</b>    | Kilogram(s)                  |
| <b>L</b>     | Litre(s)                     |
| <b>LC/NP</b> | Low Calcium/Normal Protein   |
| <b>LDL</b>   | Low Density Lipoprotein      |
| <b>LFM</b>   | Low Fat Milk                 |
| <b>LM</b>    | Lean Mass                    |
| <b>m</b>     | Meter(s)                     |
| <b>min</b>   | Minute(s)                    |
| <b>mg</b>    | Milligram(s)                 |
| <b>ml</b>    | Millilitre(s)                |
| <b>mmol</b>  | Millimole(s)                 |
| <b>ng</b>    | Nanogram(s)                  |

|                                |                                  |
|--------------------------------|----------------------------------|
| <b>nmol</b>                    | Nanomole(s)                      |
| <b>pg</b>                      | Picogram(s)                      |
| <b>PRO</b>                     | Protein                          |
| <b>RONs</b>                    | Reactive Oxygen/Nitrogen Species |
| <b>rpm</b>                     | Revolutions Per Minute           |
| <b>TCHOL</b>                   | Total Cholesterol                |
| <b>TG</b>                      | Triglycerides                    |
| <b>TNF-<math>\alpha</math></b> | Tumor Necrosis Factor Alpha      |
| <b>W</b>                       | Watt                             |
| <b>WAT</b>                     | White Adipose Tissue             |
| <b>WHO</b>                     | World Health Organization        |
| <b>wk</b>                      | Week                             |
| <b>yrs</b>                     | Years                            |

## **1. Introduction**

### **1.1 Background – What is obesity?**

Obesity is an excessive or abnormal accumulation and distribution of white adipose tissue (WAT) that has been well established as being a major risk factor for various chronic disease states [1-4]. The World Health Organization (WHO) uses the Body-Mass Index ( $BMI = \text{weight [kg]} / \text{height [m]}^2$ ) as a simple method of classifying individuals as being normal weight, overweight or obese [5]. Using this method, individuals with a BMI of 30 or greater are classified as ‘obese’. Those who are classified as being ‘overweight’, with a BMI of 25-29.9, are at a significantly increased risk of becoming obese [5], and are considered as having an increased risk of developing chronic diseases. The proportion of the world’s population that is classified as overweight or obese is increasing [4, 6-9] due to a number of different factors, including but not limited to: reduced physical activity and increased intake of food, specifically consumption of energy dense foods [3, 10]. In 2009, approximately two thirds of the adult population of the United States was considered overweight or obese [11], while approximately half of Canadian adults were considered overweight or obese [8]. Attempts have been made to prevent or reverse the growing obesity ‘epidemic’ through different strategies, such as reducing the amount of food consumed or focusing on intake of specific foods [11-22], and through increasing physical activity in the form of endurance exercise [17, 23-26]. Diet and physical activity are very important to overall health. They also appear to have important roles in influencing levels of inflammation, one of the chronic problems associated with being overweight and obese [2, 7, 10, 17, 19, 26-37]. Chronic inflammation is linked to many other health problems associated with increased body

mass and decreased physical activity [7, 11, 29, 34, 35, 37, 38]. Considering the widespread issues related to excess weight gain and the associated health problems, it is important to understand the risks and the various interventions available to counteract or even prevent this issue.

## **2. Review of Literature**

### **2.1 Increased body mass**

White adipose tissue (WAT) is the major source of stored energy in the human body [35]. Any energy-providing macronutrient consumed in excess by the body, whether protein, fat or carbohydrate, can be metabolized or stored in WAT as energy dense triacylglycerols [3, 35]. More than just being a site for energy storage, WAT is now also recognized as having the properties of an endocrine organ [2, 17, 29, 35], with visceral adipose tissue deposits being extremely active in this way [3, 9, 11, 17, 26]. Leptin and adiponectin are two examples of hormones released from WAT, along with numerous other signalling factors [35, 39]. These adipose hormones, collectively called adipokines, are suggested to have various influential effects, including: regulation of appetite, energy balance, insulin sensitivity, lipid metabolism, and whole body inflammation [4, 29, 35]. The various health problems associated with being overweight and obese, include: loss of insulin sensitivity (high blood-glucose levels at rest), dyslipidemia, hypertension, cardiovascular disease, and some types of cancer [1, 6, 7, 11, 29, 35, 36, 38, 40]. Based on these observations, the focus of much research has been on the links between adipokines and these various disorders [35, 40]. Adipokines have also been linked to the formation of ectopic fat accumulation [4, 9]. Ectopic fat is an unusual

deposit of triacylglycerols that forms in an area of the body other than adipose tissue, such as skeletal muscle or the liver, and is typically associated with development of insulin insensitivity [4, 9, 39]. Interestingly, a mechanism that has been identified as a common element among these various metabolic problems is inflammation [1, 7, 11, 29, 35, 36, 38, 41].

## **2.2 Inflammation**

Inflammation can be loosely defined as a localized phenomenon which develops due to tissue damage and is an important mechanism in the restoration of the damaged tissues [42]. However, obesity is associated with what appears to be a state of chronic whole body inflammation, that appears to be related to inflammation of WAT deposits [1, 2, 19, 26, 29, 35, 37, 41]. WAT itself is the source of many components of the typical inflammatory response, and some of the factors secreted into the circulation by WAT influence other tissues to secrete pro-inflammatory factors [29, 31, 35]. Many markers of inflammation, such as the pro-inflammatory cytokines interleukin-6 (IL-6), tumor-necrosis factor alpha (TNF- $\alpha$ ), and C-reactive protein (CRP), are found in unusually high levels in the blood of overweight and obese individuals [6, 7, 29, 35, 41]. Increased levels of these markers are commonly observed even in young overweight/obese children [33].

TNF- $\alpha$  is a pro-inflammatory cytokine that is both expressed in and released by WAT [35, 39], although it is primarily secreted by macrophages [29]. It appears to play a direct role in the development of insulin-resistance through inhibition of insulin signaling [2, 29, 35]. Elevated TNF- $\alpha$  levels have also been noted in individuals with inflammatory liver disease, and the increased TNF- $\alpha$  levels in this case are seen in conjunction with

elevated leptin levels [43]. TNF- $\alpha$  has been suggested to have a key role in the production of other cytokines, including IL-6 [29, 35], but the mechanisms underlying this influence are unclear. IL-6 not only affects WAT, but is also released into the circulation and acts on the liver, increasing the amount of hepatic CRP release [29], which in turn has a role in the onset of lipid peroxidation [44]. IL-6 has also been shown to reduce the insulin-stimulated uptake of glucose by skeletal muscle, thereby contributing to insulin resistance [45]. Adiponectin, previously mentioned as one of the influential adipokines, appears to have anti-inflammatory and antioxidant properties through inhibition of TNF- $\alpha$ . However, as opposed to other adipose-related secretions, adiponectin levels appear to be lower in overweight and obese individuals [7, 35, 39, 46].

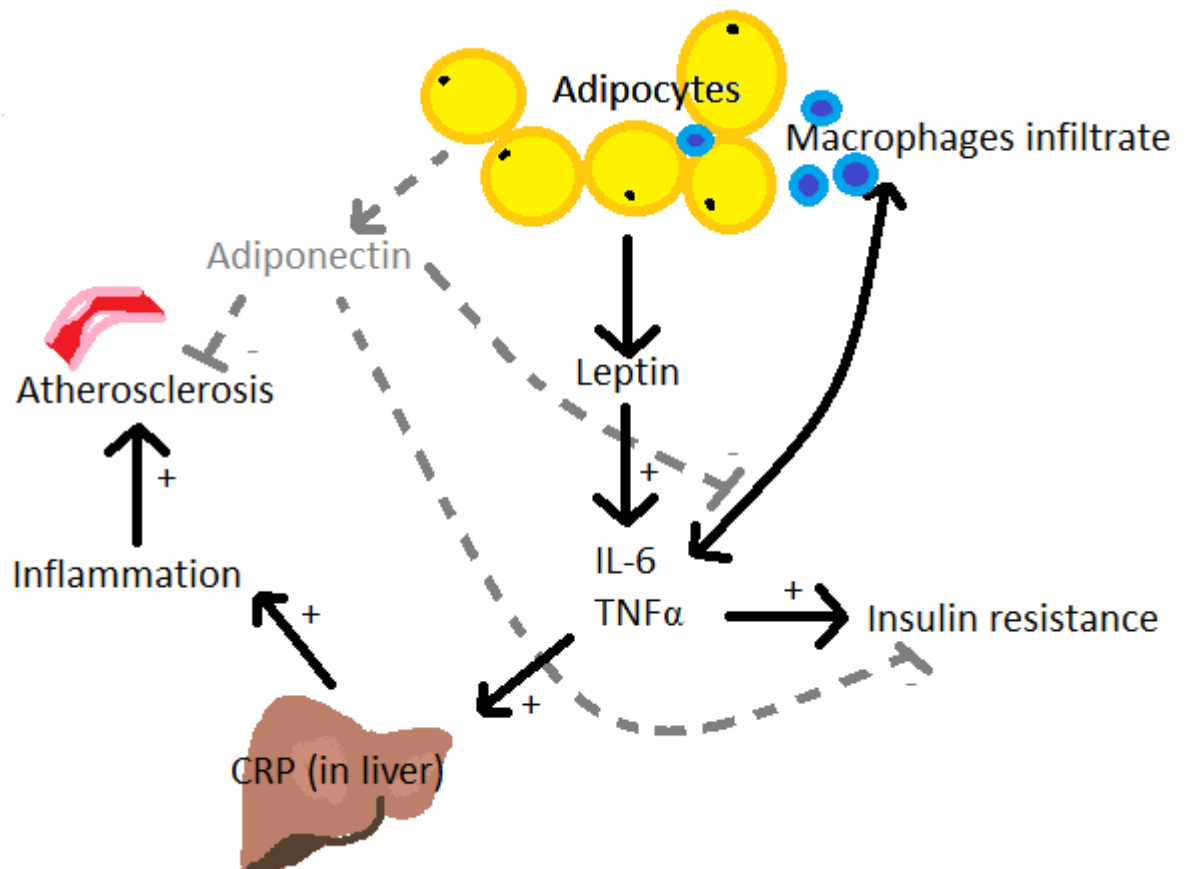
It is currently unclear what directly causes this state of chronic inflammation with obesity, although a number of possible mechanisms have been suggested. One such mechanism is that as WAT accumulates, some of the tissue within these deposits become hypoxic due to the reduced vasculature found in adipose tissue [35]. In response to localized hypoxia, these areas undergo an acute inflammatory response in an attempt to increase blood flow [35]. In brief, this process involves hypoxic inducible factor 1 (HIF-1), which becomes active under hypoxic conditions and when active, it appears to stimulate angiogenesis [47]. Another function of HIF-1 with hypoxia, is the pre-programmed cell death (apoptosis) of some adipocytes, which would further contribute to the increased inflammatory response [3, 46, 47]. As macrophages move into the area to dispose of the dead cells, they themselves give off inflammatory cytokines [3, 29, 46]. Another suggested mechanism as to why an increase in inflammation is seen with obesity may be related to exorbitant macronutrient intake, particularly of fat, which may be

causing inflammatory responses by being mistaken for an external threat [3, 32, 41]. This could be due to increased expression of immune receptors, which in turn increase the inflammatory response [32, 41].

Interestingly, other researchers have suggested that chronic inflammation causes obesity, rather than being an effect of weight gain [10, 31, 35, 48]. Some studies found that the presence of fibrinogen and other inflammatory markers, including CRP and TNF- $\alpha$ , can predict increased weight gain over a 3-year period [10, 31]. Another study suggested that increased levels of inflammatory markers begins in the womb, and it is this early exposure that can lead to obesity during childhood and adulthood [48]. Despite the lack of clarity on the sequence of events, once adiposity increases and elevated inflammatory markers are present within an individual, these factors appear to perpetuate one another and in this way are likely 'causal' of each other [28, 31, 33]. It is becoming apparent that the constant state of inflammation in turn contributes to the pathogenesis of many of the other health problems associated with being overweight or obese, including insulin resistance, atherosclerosis, oxidative stress and metabolic syndrome [19, 35, 49].



Figure 1. Proposed role of adipokine action within overweight/obese individuals, illustrated by the solid lines. The dashed lines indicate otherwise healthy functions that are inhibited in obesity. Adapted from Bastard et al. [29].



## 2.3 Interventions

Two key interventions that are commonly used to combat the increased incidence of individuals who are overweight or obese are dietary alterations and/or increased physical activity (exercise). Generally these interventions target individuals who are overweight or obese in order to induce weight loss through alterations in energy balance [8, 17, 24, 50, 51]. Other studies have used the same interventions in an attempt to indirectly treat some of the health effects of being overweight or obese, such as reducing inflammation and oxidative stress, and improving body composition [8, 17, 23, 24, 26, 44]. This review will focus specifically on dairy foods and endurance exercise and the possible role of these interventions in contributing to overall improvements in the health of individuals who are overweight and/or obese. Based on previous literature focusing on dietary changes (see Table 1) we see that the ingestion of additional dairy and calcium over a period of more than 12 weeks appears to induce benefits such as improved body composition with lower fat mass and increased lean mass, increased lipid excretion and thereby less lipid deposition, and reduced levels of cholesterol and pro-inflammatory markers. Similarly, studies that investigate the effects of exercise (see Table 2) indicate that long- and short-term exercise programs can have beneficial effects on body composition by lowering body weight and fat mass, reducing unhealthy cholesterol, reducing pro-inflammatory markers, and improving levels of glucose and insulin in the bloodstream.

Table 1. Summary of studies with dietary measures for weight change and/or health risk

| Study                      | Subjects   | Intervention   | Results  |
|----------------------------|--|--|--|
| <b>Fecal Fat Excretion</b> |  |  |  |
| Bendsen et al. [52]        | Overweight ♀♂<br>25-47 yrs<br>N = 11                 | 2 diets, short-term:<br>a) High Ca <sup>2+</sup><br>b) Low Ca <sup>2+</sup>  | More fat excretion after a) than b)  |
| Ditscheid et al. [53]      | Normal weight ♀♂<br>25 ± 2 yrs<br>N = 31             | 2 diets, short-term:<br>a) Ca <sup>2+</sup> phosphate enriched bread<br>b) placebo bread   | No difference in excretion between groups  |
| Jacobsen et al. [54]       | Overweight ♀♂<br>24 ± 2 yrs<br>N = 10                | 3 isocaloric diets, short term:<br>a) Low Ca <sup>2+</sup> , normal protein<br>b) High Ca <sup>2+</sup> , normal protein<br>c) High Ca <sup>2+</sup> , high protein          | All groups experienced increased fat excretion compared to baseline  |
| Lorenzen et al. [55]       | Overweight ♂<br>32.8 ± 1.2 yrs<br>N = 15             | 4 diets, short-term:<br>a) Low Ca <sup>2+</sup> , high fat<br>b) High Ca <sup>2+</sup> , high fat<br>c) High Ca <sup>2+</sup> , low fat<br>d) Low Ca <sup>2+</sup> , low fat | More fat excretion after the high Ca <sup>2+</sup> diets (b and c) than the low Ca <sup>2+</sup> diets (a and d)   |
| <b>Body Composition</b>    |  |  |  |
| Caron-Jobin et al. [13]    | Overweight ♀<br>47 ± 4 yrs                           | N/A, cross sectional study   | ≥2 portions dairy/d correlated with smaller adipocyte size   |
| Harvey-Berino et al. [56]  | Overweight and obese ♀♂<br>45 ± 6.6 yrs<br>N = 54    | Long-term, moderate exercise, 2 diets with calorie deficit:<br>a) 3-4 servings dairy /d<br>b) 1 serving dairy/d  | All groups lost BW and FM post-intervention<br>No significant differences between groups   |
| Jacobsen et al. [54]       | Overweight ♀♂<br>24.2 ± 2 yrs<br>N = 10              | 3 isocaloric diets, short-term:<br>a) Low Ca <sup>2+</sup> , normal protein<br>b) High Ca <sup>2+</sup> , normal protein<br>c) High Ca <sup>2+</sup> , high protein          | No BW change in any group  |
| Thompson et al. [57]       | Obese ♀♂<br>41 yrs<br>N = 72                         | Long term, 3 diets with calorie deficit:<br>a) 2 servings dairy<br>b) 4 servings dairy<br>c) same as b) with increased fiber   | No significant difference in FM or waist + hip circumference between groups  |
| Zemel et al. [58]          | Obese ♀♂<br>49 ± 6 years<br>N = 32                   | 3 diets with calorie deficit, long-term:<br>a) low Ca <sup>2+</sup> as supplement<br>b) high Ca <sup>2+</sup> as supplement<br>c) high Ca <sup>2+</sup> from dairy           | Group c) had greatest decrease in BW   |
| Zemel et al. [59]          | Obese ♀♂<br>41.7 ± 2.7 yrs<br>N = 39                 | 2 phases, long-term:<br>1: 2 eucaloric diets –<br>a) Low dairy<br>b) High dairy<br>2: as 1) but 500kcal/d deficit  | Phase 1: no significant differences in BM;<br>b) lost more trunk fat and gained more LM than a)<br>Phase 2: b) lost more BM and trunk fat, and gained more LM compared to a) |
| Zemel et al. [37]          | Overweight and obese ♀♂<br>31.0 ± 10.3 yrs<br>N = 20 | 2 diets, randomized crossover:<br>a) soy-based placebo<br>b) 3 servings dairy/d  | No significant changes in BM, FM, Trunk fat, LM  |
| <b>Blood lipids</b>        |  |  |  |
| Ditscheid et al. [53]      | Normal weight ♀♂<br>25 ± 2 yrs                       | 2 diets, short-term:<br>a) Ca <sup>2+</sup> phosphate enriched bread<br>b) placebo bread   | TCHOL and LDL lowered after CaP diet<br>No change after placebo  |

|                             |  |   |  |
|-----------------------------|--|---|--|
|                             | N = 31   |   |  |
| Steinmetz et al. [60]       | Normal weight and overweight ♂<br>20-36 yrs<br>N = 8 | 2 diets, short-term:<br>a) skim milk<br>b) whole milk                           | Lower [TCHOL] decreased by more after skim than whole milk                                 |
| <b>Inflammatory Markers</b> |  |   |  |
| Zemel et al. [37]           | Overweight and obese ♀♂<br>31.0 ± 10.3 yrs<br>N = 20 | 2 diets, randomized crossover:<br>a) soy-based placebo<br>b) 3 servings dairy/d | Dairy group had significant decreases in IL-6 and TNF- $\alpha$<br>No changes in soy group |

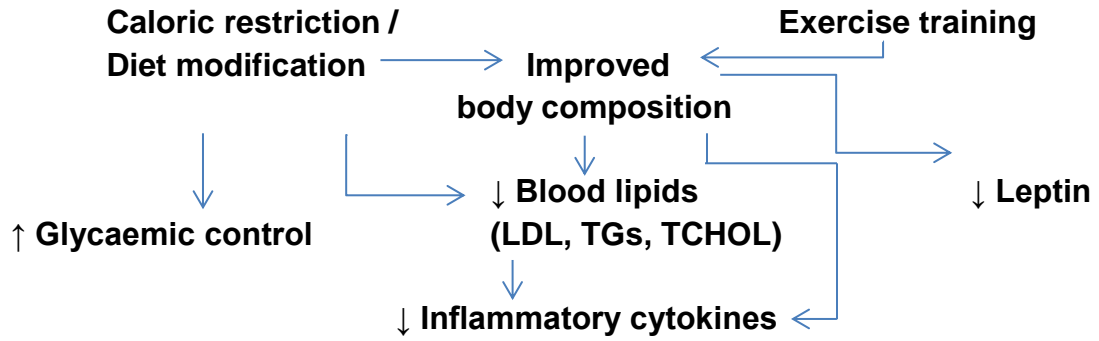
Abbreviations: ♀ - females; ♂ - males; yrs – years; short-term: less than 12 weeks duration; long-term: 12 weeks duration or more; Ca<sup>2+</sup> - calcium; DW – dry weight; x /d –per day; mo. – month; kcal – kilocalories; BW – body weight; FM – fat mass; LM – lean mass; TCHOL - total cholesterol; LDL – low-density-lipoprotein cholesterol; [x] – concentration of ‘x’; CD – control diet

Table 2. Summary of studies with exercise measures for weight change and/or health risk

| Study                       | Subjects  | Intervention  | Results  |
|-----------------------------|---|---|--|
| <b>Body composition</b>     |   |   |  |
| Carter et al. [61]          | Normal weight<br>♀♂<br>22 ± 1 yrs<br>N = 16       | Short-term progressive training on cycle ergometer<br>Low-moderate intensity                                    | Lower FM post-intervention   |
| Devries et al. [8]          | Normal weight and obese ♀♂<br>20-55 yrs<br>N = 41 | Long-term progressive training on cycle ergometer<br>Low-moderate intensity                                     | No differences due to training or gender on BW   |
| Kraus et al. [51]           | Overweight ♀♂<br>52.3 ± 7.8 yrs                   | Long-term, 3 activity levels:<br>a) sedentary<br>b) high intensity<br>c) moderate intensity<br>d) low intensity | Lower BW post-intervention in b) and d) compared to a)   |
| McKenzie et al. [62]        | Normal weight<br>♀♂<br>26.9 ± 3.4 yrs<br>N = 14   | Short-term training protocol on cycle ergometer<br>Moderate intensity   | Lower FM post-intervention   |
| Miyazaki et al. [63]        | Normal weight ♂<br>19-21 yrs                      | Long-term training protocol, running<br>High intensity  | No difference in BW or FM post-intervention  |
| <b>Blood lipids</b>         |   |   |  |
| Kraus et al. [51]           | Overweight ♀♂<br>52.3 ± 7.8 yrs                   | Long-term, 3 activity levels:<br>a) sedentary<br>b) high intensity<br>c) moderate intensity<br>d) low intensity | No difference in LDL to TCHOL ratio between groups<br>Lower LDL particle size in b), c) and d) than a) |
| <b>Metabolic markers</b>    |   |   |  |
| Bruce et al. [64]           | Obese ♀♂<br>36 ± 3 yrs<br>N = 9                   | Short-term training on cycle ergometer<br>Moderate intensity  | Lower plasma insulin post-intervention   |
| Devries et al. [8]          | Normal weight and obese ♀♂<br>20-55 yrs<br>N = 41 | Long-term progressive training on cycle ergometer<br>Low-moderate intensity                                     | No differences in metabolic markers post-intervention  |
| Devries et al. [44]         | Normal weight and obese ♀ age<br>20-50<br>N = 24  | Long-term progressive training on cycle ergometer<br>Low-moderate intensity                                     | No difference in fasting glucose or insulin post-intervention  |
| <b>Inflammatory markers</b> |   |   |  |
| Bruce et al. [64]           | Obese ♀♂<br>36 ± 3 yrs<br>N = 9                   | Short-term training on cycle ergometer<br>Moderate intensity  | Lower plasma adiponectin post-intervention   |
| Donges et al. [65]          | Overweight ♀♂                                     | Short-term training protocol<br>Moderate intensity  | Lower CRP<br>Trend for lower IL-6  |

Abbreviations: ♀ – females; ♂ – males; yrs – years; short-term: less than 12 week duration; long-term: 12 week duration or more; low intensity: 50% VO<sub>2peak</sub> or less; moderate intensity: 50-75% VO<sub>2peak</sub>; high intensity: 75% VO<sub>2peak</sub> or more; wk – week; mo. – month; min – minutes; mi/wk – miles per week; BW – body weight; FM – fat mass; TCHOL - total cholesterol; LDL – low-density-lipoprotein cholesterol; CRP – C-reactive protein; IL-6 – interleukin-6

Figure 2. Proposed mechanisms through which lifestyle interventions may attenuate obesity-related risk factors. (Adapted from Vincent et al. [6].)



### *2.3.1 Bovine milk components and body composition*

In the past, dietary intake of bovine milk had been generally assumed to cause weight gain due to its high energy and fat content [66]. However, more recent research indicates that individuals who consume high amounts of dairy typically lose weight when dieting, and those who have successfully lost weight do not experience weight regain when consuming dairy [11, 21]. There is increasing evidence to show that consumption of dairy foods and dietary calcium is inversely related to obesity and is associated with positive effects on weight loss (see Table 1) [11-14, 19, 20, 36, 55]. Individuals who consume high amounts of dairy foods are at a 67% lower risk of gaining weight over the course of 10 years [11]. Researchers have looked at both human and animal models to determine whether calcium supplements alone are enough to elicit these beneficial effects or whether dairy, as a ‘whole food’, is preferable for improvements in body composition. For example, mice given a high calcium diet in the form of low fat dry milk had significantly reduced body weight when compared to mice on a control diet and mice on a high-calcium diet devoid of milk [19, 36, 67]. These findings suggest that, in mice at least, calcium-intake-induced weight loss is associated with milk as a whole food, rather than calcium as a stand-alone component. Similar findings have also been observed in human subjects [11, 12, 16, 20]. Furthermore, increased calcium intake via dairy significantly reduced the amount of post-prandial lipid found in the blood while calcium supplementation alone did not [16]. Collectively, these observations suggest that other components of milk likely contribute to the weight-loss effect, possibly acting alone or in combination with calcium. These beneficial components of milk, including calcium, are collectively known as bioactive compounds, and are released through digestive processes

[21, 68]. Bioactive compounds can be defined as components of whole foods that may have regulatory effects beyond basic nutrition [68].

It should be noted that there is also evidence to show that increased intakes of calcium alone have beneficial effects on weight loss and body composition. Calcium intake of approximately 800mg per day appears to be the threshold for body composition benefits [11], but calcium intake via milk/dairy intake appears to be the most beneficial [11, 12, 16, 20]. According to the Dairy Farmers of Canada, a glass of milk contains about 300mg of calcium [69]. Therefore, drinking three servings of milk a day would provide the necessary calcium to achieve this benefit for body composition, and this is not taking into consideration any other dairy intake [11]. The mechanisms underlying how dairy calcium intake relates to weight loss and body composition have yet to be fully determined, although many different theories have been proposed. When dairy calcium is increased in mice this may prevent or reduce adiposity through interaction of calcium and lipids in the digestive tract, where high intestinal calcium concentrations lead to binding of calcium and fatty acids, creating ‘soaps’ which are indigestible [19, 70]. These soaps in turn lead to more lipid being excreted [19] leaving less lipid available for absorption and storage in adipose tissue. Interestingly, these mice were also more responsive to post-prandial insulin secretion [19]. Similar responses have been seen in human subjects, where a diet high in dairy calcium increases the amount of fat excretion when compared to low dairy calcium diets (see Table 1) [11, 16, 21, 55]. Furthermore, increases in insulin sensitivity were also observed with the increased intake of dairy calcium [27]. Increased dairy-calcium intake has been shown to decrease low-density-lipoprotein (LDL) cholesterol (see Table 1) [55, 60, 66, 71, 72], potentially through excretion, indicating



that increased lipid secretion may be a possible mechanism for preventing or reversing elevated blood cholesterol. High calcium intake has also been negatively associated with high plasma lipids and lipoproteins [53, 66, 73].

There is also evidence to suggest that the hormone calcitriol (1,25-dihydroxyvitamin D) is inhibited by elevated dietary calcium intake [12, 13, 19, 36]. Calcitriol is perhaps most commonly known for its actions within the intestines and kidneys to promote absorption of calcium and phosphorus, and in bone metabolism regulation [74]. However, there is evidence to suggest that calcitriol also acts upon vitamin D receptors present in adipose tissue; when stimulated, these receptors act to increase intracellular calcium concentrations ( $[Ca^{2+}]_i$ ) [20, 21, 67, 75]. When  $[Ca^{2+}]_i$  is high, lipogenesis is promoted through stimulation of fatty acid synthase, and lipolysis is inhibited [12, 13, 36]. When dietary calcium levels are high, calcitriol action upon adipose tissue is inhibited, thereby lowering  $[Ca^{2+}]_i$  and promoting lipid mobilization and reduction of adipose depots [12, 13, 36, 67, 75]. It is unclear if increased consumption of vitamin D-fortified dairy affects these processes. Taking these calcium-related mechanisms together, high dairy intake may reduce the overall amount of energy stored as white adipose tissue by increasing the amount of lipid excreted, reducing the amount of lipid absorbed and possibly reducing intracellular lipogenesis.

Calcium is not the only bioactive component of milk that has health benefits. Other bioactive compounds include the peptides caseokinins and lactokinins, which are created through the digestion of the milk proteins casein and whey [32, 68]. These particular bioactive peptides have been observed to have ACE (angiotensin-I-converting enzyme)-inhibitory effects [32, 66, 68, 76]. ACE has many functions, perhaps most

notably regulating blood pressure [38, 68, 76]. When left to function normally, ACE converts angiotensin-I into angiotensin-II, which is a vasoconstrictor. However, when ACE is inhibited, angiotensin-II is not formed which prevents the hypertensive effect, while the action of bradykinin is enhanced [38, 68, 76]. Bradykinin is a vasodilator, and the overall effect of ACE inhibition is the lowering of blood pressure [38, 66, 68, 76]. Angiotensin-II is also involved in increasing the expression of adipocyte fatty acid synthase, an enzyme which aids in fat deposition in WAT [12, 13, 20, 21]. In this way, dairy-based ACE inhibition has been suggested to be involved in metabolism regulation and may have a mild anti-obesogenic effect [12, 16, 20, 21, 38].

Branched-chain amino acids (BCAAs) are another bioactive component of milk that appears to have anti-obesity effects [19-21, 77]. BCAAs are found in significant quantities in the milk proteins whey and casein, and they play a key role in protein metabolism and muscle protein synthesis [14, 20, 21, 66]. Because some of the proteins found in milk are 'slow' proteins – meaning they are slow to be digested and absorbed in the intestine – these proteins are present in the bloodstream for a prolonged period of time due to gradual intestinal uptake, providing a more sustained supply of amino acids for more effective muscle protein synthesis [66, 77-79]. Therefore, dairy represents a quality nutritional choice to fuel protein synthesis, and facilitating an enhanced protein balance.

Considering the numerous beneficial components found within bovine dairy, it is important to establish how much dairy should be ingested to optimize the potential health benefits. Considering calcium alone as a bioactive compound in milk, it is well established that 3 servings (3 x 250 mL) of milk per day provides enough calcium to surpass the threshold of 800mg/day, considered to be optimal for body compositional

benefits [11, 69]. Looking at milk as a ‘whole food’, provision of three servings of dairy products per day over a span of 6 months has been shown to significantly reduce body weight when compared to a control group and a calcium-supplemented group ( $11.1 \pm 1.6\text{kg}$  lost vs.  $6.6 \pm 2.6\text{kg}$  and  $8.6 \pm 1.6\text{kg}$ , respectively) [58]. Three servings of dairy products per day have also been shown to reduce circulating TNF- $\alpha$ , IL-6 and CRP levels (~15%, 13% and 57%, respectively) over 28 days [37]. Another study compared two types of dairy products, looking at 2.5 servings daily of skim milk as opposed to whole milk, and found that after 6 weeks skim milk resulted in greater reductions in blood lipids and total cholesterol [60]. Taken together, these studies suggest that consumption of approximately 3 servings of dairy product per day, and skim milk in particular, appear to be effective for improvements in body weight and blood markers of inflammation, both contributing to overall health.

In summary, bovine milk products contain many components that appear to be beneficial for health management. Milk contains components that contribute to weight loss and management, such as BCAAs, which are related to increases in lean body mass [14, 20, 21, 37, 66], and calcium, which increases fatty acid excretion and inhibits adipose accumulation [11-14, 16, 19-21, 36, 55]. In addition, milk contains nutrients which act to prevent or reduce the negative effects that are associated with being overweight or obese. These include hypertension and high cholesterol, and reductions in both of these negative factors have been associated with bovine milk components [32, 55, 60, 66, 68, 71, 72, 76]. Taken together, bovine milk appears to be beneficial for weight management and the associated health problems when consumed in adequate amounts in humans.

### 2.3.2 Bovine milk components and inflammation

Apart from weight loss, dairy foods and components have also been investigated in regards their effects on whole body inflammation and oxidative stress. In mice, high calcium intake in the form of low fat dry milk reduced inflammation, both systemically and more specifically in WAT [19, 36]. A possible explanation for this effect is that gene expression of inflammatory cytokines is inhibited when dairy is consumed, and TNF- $\alpha$  activity is suppressed [36]. Dairy consumption also results in increased amounts of adiponectin [2, 36], which, as previously stated, has anti-inflammatory effects. Dietary intake of the dairy fatty acids pentadecanoic acid and heptadecanoic acid are negatively associated with the inflammatory marker C-reactive protein (CRP) [7]. However, due to the very small amounts of these fatty acids present ( $0.21 \pm 0.04\%$  total phospholipid fatty acids present in milk) [7] it is unknown whether this effect is due to the fatty acids themselves or dairy intake in general.

In humans, there is mixed evidence for dairy and dairy compounds having an effect on inflammation (see Table 1). In one study looking at supplementation with whey protein, the researchers noted no changes in TNF- $\alpha$ , IL-6 and CRP in obese individuals (BMI =  $31.3 \pm 0.8$ ) [32, 80], whereas a different study observed no changes in inflammatory markers with casein supplementation [73]. Other studies indicate that dairy intake reduces whole-body inflammation and inflammation associated with various disease states such as insulin insensitivity/diabetes [27, 73, 81]. The discrepancies between these studies appear to be primarily due to the difference between supplementation with milk components and consumption of milk as a ‘whole food’ – the studies which saw no changes in inflammatory markers focussed on milk proteins

ingested as supplements, while the studies which did observe positive changes consumed milk. Based on these observations, investigation into the role of dietary intake of milk as a whole food represents an important area of study.

### *2.3.3 Exercise and body composition*

Endurance exercise is a common intervention used for maintenance of a healthy body weight. Health Canada defines regular endurance exercise as 30-60min per day of light-moderate exercise at 50-65% of maximal heart rate (HRmax), approximately 5 days per week [82]. There is a negative correlation between amount of physical activity and measures of body fat – as physical activity increases, measures such as BMI and waist circumference decrease (see Table 2) [39]. Research indicates that individuals who participate in even moderate to low intensity activity (the caloric equivalent of walking 8-12 miles per week at 40-50% peak oxygen consumption) experience weight loss in comparison to sedentary controls, suggesting that any amount of activity is beneficial for achieving healthy weight status [51].

The risk of developing various diseases, such as diabetes, cardiovascular disease, hypertension and certain cancers, is greatly decreased in individuals who engage in regular physical activity [50], and it can prevent or reduce excessive adiposity [8, 50]. In regards to diabetes, engaging in regular physical activity/exercise results in a reduction of resting plasma insulin levels [64], indicating that less insulin is required to elicit a reduction in blood glucose (increased insulin sensitivity), and may also contribute to decreasing the chances of developing insulin resistance and eventually type II diabetes. This effect appears to be independent of weight loss [64]. There is also a general consensus that regular exercise can favourably decrease the amount of circulating lipids

in the bloodstream [23, 83]. Furthermore, engaging in long-term endurance exercise has been shown to increase concentrations of HDL cholesterol, while exercise-induced weight loss can reduce unhealthy cholesterol and the risk associated with high cholesterol levels [23, 83]. This effect is likely due in part to an exercise-induced change in the blood distribution of lipoproteins, as research indicates that long-term exercise results in a shift in the distribution towards higher density lipoprotein particles that are less likely to block arteries [23, 51]. These observations suggest that exercise is linked to reduced risk of diseases associated with high adiposity and BMI.

In addition to reduced adiposity and disease risk, exercise contributes to healthy body composition through increased energy turnover. Endurance training reduces reliance on both carbohydrate, in the forms of glucose and muscle glycogen, and amino acids as fuels, and increases the reliance on lipids as a source of fuel for muscle activity [61, 62, 64, 84] for a given absolute intensity. As a fuel for activity, lipids come from within the muscle itself (intramuscular triglycerides) and are also mobilized from adipose tissue [25]. Increased use of lipids as fuel maybe partially mediated through healthy circulating adiponectin levels [64], which have been shown to improve with decreased body weight and increased dairy intake [2, 36].

In summary, participation in regular exercise results in various adaptations that relate directly to body composition and energy metabolism. Furthermore, these adaptations appear to be beneficial not just for body weight management, but for also for reduction in disease risk.

#### *2.3.4 Exercise and inflammation*

Acute bouts of exercise; whether endurance or resistance, maximal or submaximal, have all been shown to result in an increase in inflammation after activity cessation (see Table 2) [2, 17, 85]. There is debate as to whether this inflammatory response is due to muscle tissue damage as a direct result of exercise, or due to damage caused by increased reactive oxygen/nitrogen species (RONS) production [85]. However, there is evidence to indicate that long-term training protocols result in reduced inflammatory markers at rest, indicating a beneficial effect of exercise [2, 17, 26, 44]. There are a few possible mechanisms for the reduction in basal inflammatory markers. One possibility is through the reduction of adipose tissue typically seen with long-term exercise: as the volume of adipose tissue decreases, the amount of inflammatory cytokines secreted also decreases, resulting in an overall reduction in whole body inflammation [17, 44]. Interestingly, regular participation in exercise has been observed to reduce CRP levels in the blood even if there were no changes in body mass or body composition [26], indicating that there may also be a weight-independent mechanism. Furthermore, exercise induces release of IL-6 and up regulates IL-6 mRNA in skeletal muscle [39]. Although IL-6 is considered to be a pro-inflammatory marker, when it is released during exercise it acts as an anti-inflammatory agent, and also inhibits the effects of TNF- $\alpha$  [39]. Taken together, this evidence indicates that repeated exercise participation (training) is a useful method of reducing inflammatory markers and increasing anti-inflammatory agents.

#### *2.3.5 Bovine milk and exercise in combination*

As previously discussed, weight loss - whether due to increased physical activity or dietary interventions - has the beneficial effect of reducing whole-body inflammation

and inflammatory markers [2, 31]. Considering that both bovine milk and aerobic exercise produce this beneficial effect independent of one another, the natural next step is to investigate their combined effects and determine if their effects are additive.

When increased dairy consumption is combined with long-term endurance exercise, there appears to be an effect of reducing muscle catabolism and encouraging muscle protein anabolism, facilitating gains in lean muscle [86-88]. These results indicate that protein, specifically BCAAs, are an important component of exercise-related nutrition, and it has been suggested by some that they should be included in any foods or beverages consumed for exercise preparation and recovery [87, 88]. Other studies have found increased insulin sensitivity and reduced inflammation in response to the combination of dietary and exercise interventions in individuals who are obese [6, 88]. ACE inhibition, as has been previously outlined to be one of the possible benefits of dairy consumption, has also been shown to improve endurance exercise capacity in rats [89]. Interestingly, many high-level endurance athletes have a natural inhibition of ACE due to genetic traits [89-91], suggesting that ACE inhibition may facilitate endurance capacity.

Milk also has many components that are important for endurance exercise recovery, including carbohydrates and proteins previously mentioned, as well as electrolytes, vitamins and minerals [66, 77, 92]. Milk protein is important in exercise recovery to provide a more prolonged elevation of blood amino acids, possibly facilitating protein synthesis in muscle [77]. Some work has shown that consuming chocolate milk (1% milk fat) in conjunction with exercise increases fatty acid circulation in the blood, which may allow for individuals to engage in exercise for a longer period of time due to the higher readily available fuel, which in turn will prolong the beneficial



effects of acute exercise [84, 88]; however, this effect is not present when consuming low fat or skim milk. Milk also serves to replenish fluids and electrolytes lost as sweat during exercise, maintaining healthy fluid balance throughout exercise (when ingested prior to and during activity) and during recovery from exercise (when ingested immediately post-activity) [77, 84, 92, 93]. Milk as a post-exercise beverage has also been observed to blunt the muscle damage response to acute exercise [94, 95]. Muscle damage can occur when an individual undertakes any form of unhabituated activity, whether it be beginning a new exercise protocol, changing a habitual exercise routine, or simply shovelling snow for the first time that season, and the probability and severity of damage increases with intensity and duration of activity [96]. Suggested causes of muscle damage also include substrate depletion and RONS within the muscle [96]. Damage is characterized by edema and increased levels of myoglobin and creatine kinase in the blood, and can lead to transitory pain and decreased strength and function [95, 96]. The specific mechanisms underlying this possible role in the prevention of muscle damage remains unclear; however, one theory that has been suggested is that milk intake possibly blunts these effects due to adequate provision of amino acids needed to replace the proteins degraded due to activity, and provision at the time of need [95]. Clearly, further research is needed to better understand the role of milk intake and muscle damage following physical activity.

There is a plethora of research regarding the benefits of resistance training for overweight and obese individuals, both with and without dietary interventions. One review paper noted that the overall trend of current research indicates a beneficial effect in weight management, lean mass gains, and reduced inflammatory markers due to

resistance training [26]. There are conflicting reports regarding the efficacy of dairy supplementation in conjunction with resistance training for improved health in both athletes and sedentary populations [97-100]. However, for acute anaerobic exercise, dairy has been shown to have benefits for blunting muscle damage and associated soreness, as well as being effective at contributing to rehydration and replenishment of substrates used during exercise [77].

### 2.3.6 *Timing of nutritional intervention*

It is well established that there are numerous benefits associated with dairy intake for body composition and other health considerations. Considering this, it is important to determine whether the timing of dairy intake is an important factor when coupled with endurance exercise training. Determining the timing of intake is an important component of these interventions in order to maximize any potential health benefits and adaptations.

Much of the research into this area indicates that nutritional intake within 60 minutes of exercise cessation – typically within the first hour – has benefits ranging from optimization of muscle glycogen resynthesis, reduction of muscle protein degradation as well as improved muscle protein synthesis [101, 102]. In a study that looked at intake of a beverage containing carbohydrate, protein and fat at breakfast versus immediately post-exercise, the group who received their drink post-exercise showed improved recovery when compared to those who took their beverage at an alternate time [101]. Interestingly, this beverage also offset the drop in body mass associated with an increase in activity, which was seen in the control group [101]. A similar study, using a carbohydrate-protein supplemental beverage, found that muscle damage was attenuated to the greatest extent by consuming the beverage immediately post-exercise [87]. In contrast, a study looking

at intake of protein from various sources, including skim milk, there were no differences in the availability of amino acids in the blood when comparing intake from rest or post-exercise [103]. However, these studies all focused on individuals who are already active and so their results cannot easily be extrapolated to indicate how nutrient timing would affect overweight and inactive individuals, whether for exercise recovery or for any other benefits.

### **3. Statement of the Problem**

Considering the research on exercise and dairy products, alone and in combination, on weight management and/or inflammation, there is a growing amount of evidence to indicate that these interventions alone are likely effective strategies to possibly attenuate or even alleviate these problems. However, there are gaps in the literature when it comes to research looking at the influence of combining these interventions and determining the outcomes on risk factors. While many studies have investigated the effects of dairy on inflammation and blood lipids in combination, or dairy and exercise for weight loss, to the author's knowledge there have been no investigations into the effect of combining increased low fat dairy intake and endurance exercise on weight loss, inflammation, blood lipids and metabolic markers in overweight individuals. The exercise-based research typically involves resistance exercise, with and without milk, which does provide some insight into general exercise benefits, but there is not as much research looking at endurance exercise for these health factors, and particularly little research looking at endurance exercise and increased milk intake. Research that does utilize milk intake with endurance exercise training typically investigates milk for acute recovery rather than for long-term health. Until endurance training and increased dairy intake are studied in these contexts, our understanding of the health benefits and mechanisms remain incomplete. This is an important area investigation due to the growing incidence of obesity. Determining the effects of endurance training and dairy intake in overweight individuals is critical as endurance exercise represents a form of activity that leads to greater total amounts of energy turnover, as compared to resistance exercise, and combined they may represent simple,

cost effective and relatively easy lifestyle modifications that could work synergistically to benefit this population, helping to prevent obesity.

#### **4. Purpose**

The purpose of this proposed study was to increase both daily physical activity, in the form of endurance exercise training, and dairy intake, and to determine the combined effects of these factors. Furthermore, we wanted to determine if the timing of intake of milk is critical in facilitating enhanced adaptations. More specifically, our measures include changes in body composition,  $\text{VO}_2\text{peak}$ , blood lipids, metabolic markers, and whole body inflammation, resulting from these interventions.

## 5. Hypothesis

Overall, we hypothesized that combining increased low fat milk (LFM) intake with endurance exercise training would augment the physiological and health-related adaptations that would be experienced with either intervention alone. We hypothesized that these adaptations would include: improved body composition, with decreased body fat and increased lean mass; improved blood-lipid measures; improved metabolic measures; and decreased whole body inflammation.

Specifically, we hypothesized that three servings of LFM consumed immediately after endurance exercise cessation would lead to augmented adaptations when compared to: a) LFM consumption 4 hours before or 6 hours after endurance exercise participation; b) consumption of an isoenergetic carbohydrate beverage within an hour of exercise cessation; or c) consumption of an isoenergetic carbohydrate beverage either 4 hours before or 6 hours after exercise participation. These augmented adaptations were hypothesized as follows:

- A) Mild weight loss, or in absence of weight loss, a shift toward lower FM and higher LM,
- B) Reduction of whole body inflammation and inflammatory markers and associated health risk markers as follows:
  - a. Reduced blood lipids – total cholesterol, HDL, LDL and triglycerides
  - b. Lower metabolic markers at rest – insulin, glycogen, and leptin

As a secondary investigation, we wanted to determine if the groups ingesting milk, and the resulting elevated intakes of both calcium and vitamin D, would exhibit

correspondingly higher levels of circulating serum-25(OH)D in comparison to those ingesting the carbohydrate beverage within the time frame of the current investigation. We also anticipated that all groups would experience increases in  $\text{VO}_{2\text{peak}}$  measures due to training.

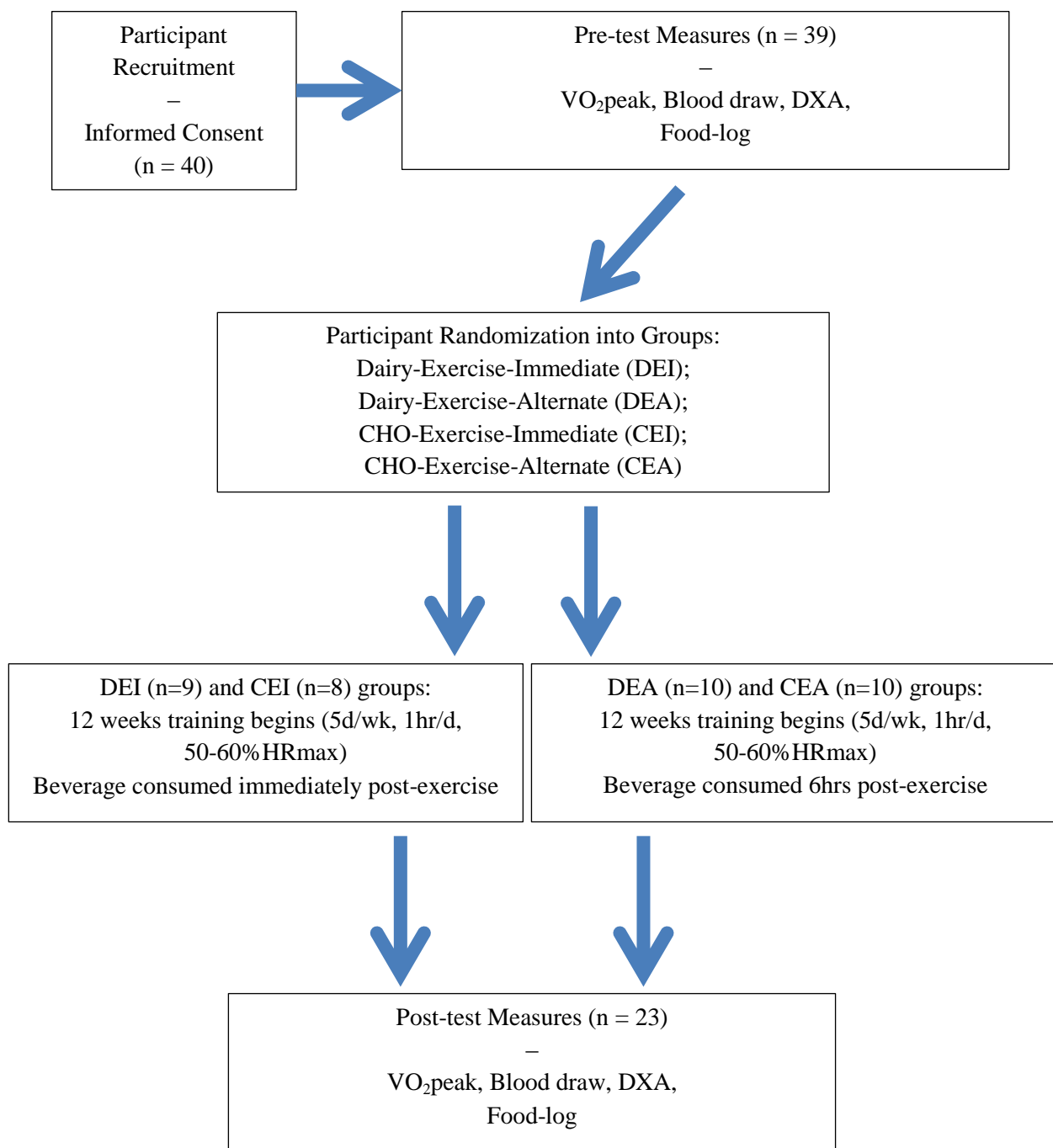


## **6. Methods**

### **6.1 Participant recruitment**

We recruited 40 untrained university-aged males whose BMI fell in the range of 22-33 (refer to Table 3 for participant baseline characteristics). This was accomplished through advertising on Brock University campus through posters, word of mouth and on the Brock University webpage. Interested individuals contacted the study organizers and an initial interview was scheduled, during which time individuals were provided with an informed consent form which outlined in detail the purpose and methods of the study. This information was also provided verbally. Interested individuals were asked to complete a medical history, a dairy intake questionnaire to confirm habitual intake, and a physical-activity readiness questionnaire (PAR-Q) to confirm applicability for exercise involvement. Their height and weight were taken to determine BMI. Participants were accepted into the study if they had no medical conditions that would prevent them from engaging in regular exercise, were not allergic to dairy products, did not exercise regularly and did not consume more than three servings of dairy products per day.

Figure 3. Outline of experimental protocol.



Abbreviations: DXA – Dual X-ray absorptiometry; d/wk – days per week; hr/d – hour per day; HRmax – maximal heart rate; hrs - hours

## 6.2 Pre-testing measures

Once accepted into the study, participants returned to the lab to complete a maximal oxygen consumption test on a cycle ergometer, to determine  $\text{VO}_{2\text{peak}}$ . This test involved a 2-minute warm up at 80W, after which the test started; the participant cycled for 2 more minutes at 80W, and then the wattage was increased by 20W every 2 minutes. This continued until exhaustion or until cycling cadence dropped below 60rpm and the respiratory exchange ratio was above 1.12; at this time the maximal oxygen consumption was taken as  $\text{VO}_{2\text{peak}}$ . Verbal encouragement was given throughout this test. Participants also completed a 4-day food log (3 week days and one weekend day) in order to estimate regular dietary patterns and verify daily dairy consumption. Food logs were analyzed using dietary analysis software (Nutritionist Pro, Version 2.2, First DataBank Inc., San Bruno, CA, USA). Participants were then randomized into one of four experimental groups, insuring that all groups were matched for clinical BMI range (20-24.9, 25-29.9, 30-34.9). The four experimental groups were: Dairy-Exercise-Immediate (DEI), Dairy-Exercise-Alternate (DEA), Carbohydrate-Exercise-Immediate (CEI), and Carbohydrate-Exercise-Alternate (CEA). Participants were blinded to the purpose of the study being an investigation of dairy intake, having been told that we were investigating ‘sports beverages’, and were also blinded as to which experimental group they were randomized into.

On subsequent lab visits, a fasted blood sample was taken and a dual X-ray absorptiometry (DXA, Lunar, GE) scan was completed. DXA scans were used to determine body composition. Fasting blood samples were collected from the antecubital vein into Vacutainer© tubes containing sodium heparin for plasma collection, and into

untreated tubes for serum collection. Plasma tubes were placed on ice, centrifuged at 2500 g for 15 min at 5°C, and aliquoted into 1.5ml tubes and stored at –86°C for subsequent analysis. Serum tubes were allowed to stand at room temperature until clotted, centrifuged at 2500 g at 5°C for 15 min, aliquoted into 1.5ml tubes and stored at –86°C for subsequent analysis.

### **6.3 Training protocol and post-testing measures**

Once all the pre-testing measures were completed, participants engaged in an endurance exercise training program on stationary bicycle ergometers (Monarch Ergomedic 828 E, Sweden), for one hour each day, five days a week for 12 weeks. This exercise took place at the Brock Research and Innovation Centre. During the first three weeks, participants cycled at an intensity of 50% their max heart rate (as determined during the VO<sub>2</sub>peak test), and then at 60% for the remaining 9 weeks. These relative heart rates were chosen to ease the participants into exercising, rather than asking sedentary individuals to begin training at a higher heart rate and intensity. Senior undergraduate research assistants who had experience in exercise prescription and personal training directly supervised all training to ensure the protocol was followed and performed at the correct workloads for the set amount of time. Different research assistants were responsible for managing beverage provision and administration. Participants in the DEI group were given 750mL of LFM (3 additional dairy servings per day), which was consumed immediately after exercise (complete consumption within 60min of training cessation). The DEA group also received 750mL of LFM, but to be consumed at an unrelated time of day. The CEI group received an isocaloric carbohydrate (maltodextrin)

beverage to be consumed within the hour following exercise. Participants in the CEA group were asked to consume their carbohydrate beverage at an unrelated time of day. Participants in the DEA and CEA groups were given the choice of consuming their beverages either 4 hours prior to their training or 6 hours after training. All individuals opted to consume their beverages 6 hours after exercise cessation. Both beverage types were the same volume (750mL) and contained approximately the same amount of energy – dairy groups consumed approximately 270kcal and carbohydrate groups consumed approximately 240kcal per day. Participants were blinded to their groups and asked not to discuss their beverages with other participants. To aid the blinding process, all drinks were flavoured with non-caloric vanilla flavouring and were administered in opaque bottles. Participants were encouraged to maintain consistent dietary patterns throughout the study period. During the last week of training, participants were asked to complete another 4-day food log to determine if any changes in habitual nutritional intake had occurred over the course of the study.

Once the twelve weeks of training were completed, participants underwent the same tests that were administered prior to training. All post-testing measures began within the last week of training.

## 7. Analysis

Body composition data obtained before and after training from DXA were compared, as well as absolute and relative  $\text{VO}_2$  peak results and maximal heart rate.

Blood lipid analysis, including total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglycerides (TGs) concentrations, were analyzed in serum samples to examine blood lipid profile alterations. This analysis was performed by the clinical chemistry lab at McMaster University, Hamilton, Ontario.

Serum samples were used to determine changes in IL-6, glucose, insulin, TNF- $\alpha$ , CRP and leptin using Millipore Milliplex® kits and MagPix software, and vitamin D using an ELISA kit (ALPCO™ Immunoassays, Salem, NH).

The graduate students running the study performed the majority of the analyses, and as such, blinding during this process was not possible. However, the technicians assisting the graduate students with the analyses were not involved with the training portion of the study and therefore blinded to participant groupings. The blood-lipid analyses were the only analyses that were truly double-blinded, as the McMaster technicians performing these analyses were only given the participant numbers for the serum being analyzed and had no other contact with the study.

As an estimate of insulin resistance, we calculated the homeostatic model assessment (HOMA) index, which is defined as the product of the fasting plasma insulin level ( $\mu\text{U/mL}$ ) and the fasting plasma glucose level ( $\text{mmol/L}$ ), divided by 22.5 [104]. In order to have serum glucose values to use in this calculation, glucose was determined using a colorimetric assay (Cayman Chemical Co., Ann Arbor, MI).

All data were analyzed using a three way-ANOVA (beverage type, intake time, pre-test/post-test) with repeated measures. A Tukey's Honest Significant Difference test was used to establish pair-wise differences when significant interactions were observed ( $p \leq 0.05$ ). A two way-ANOVA (beverage type, intake time) was also performed using delta values, which were determined by subtracting the pre-test value from the post-test value for each outcome variable.

Results are expressed as mean  $\pm$  standard error, unless otherwise stated. Statistical analyses were performed with Statistica software for Windows, version 5.0 (StatSoft, Inc., Tulsa, OK).

## **8. Results**

### **8.1 Baseline**

Forty participants enrolled in the study, and twenty-three participants completed the entire protocol. Data from one participant was excluded from statistical analysis due to pulmonary illness at the time of post-testing, leaving a final n of 22. The baseline characteristics for the participants who completed the study are described in Table 3. There were no significant differences between the different experimental groups at baseline.



Table 3. Baseline characteristics for individuals who completed the study, by group

|   | DEI (n=7)  | DEA (n=5)  | CEI (n=6)  | CEA (n=4)  | p-value |
|---|------------|------------|------------|------------|---------|
| <b>Subject Characteristics:</b>           |            |            |            |            |         |
| Age (yrs)                                 | 23.7 ± 2.5 | 20.6 ± 2.5 | 24.4 ± 3.0 | 29.4 ± 3.5 | 0.319   |
| Mass (kg)                                 | 79.5 ± 3.5 | 83.5 ± 5.6 | 90.6 ± 6.0 | 86.2 ± 4.2 | 0.422   |
| Absolute VO <sub>2</sub> peak (L/min)     | 3.7 ± 0.2  | 3.3 ± 0.2  | 3.9 ± 0.4  | 3.1 ± 0.4  | 0.540   |
| Relative VO <sub>2</sub> peak (ml/kg/min) | 46.9 ± 1.9 | 40.0 ± 5.0 | 43.8 ± 7.5 | 35.3 ± 4.0 | 0.816   |
| <b>Habitual Diet:</b>                     |            |            |            |            |         |
| Daily Energy Intake (Kcal)                | 2845 ± 424 | 2215 ± 297 | 2373 ± 531 | 2641 ± 395 | 0.275   |
| %Carbohydrate                             | 52.8 ± 1.6 | 54.8 ± 5.6 | 46.0 ± 3.  | 40.5 ± 2.5 | 0.358   |
| %Fat                                      | 30.7 ± 2.0 | 31.0 ± 5.2 | 35.9 ± 2.7 | 41.1 ± 4.5 | 0.560   |
| %Protein                                  | 14.4 ± 0.9 | 14.2 ± 1.9 | 17.3 ± 1.1 | 18.5 ± 2.0 | 0.694   |

Abbreviations: DEI – Dairy-Exercise-Immediate group; DEA – Dairy-Exercise-Alternate group; CEI – Carbohydrate-Exercise-Immediate group; CEA – Carbohydrate-Exercise-Alternate group; yrs – years; kg – kilograms; ml/min – millilitres per minute; ml/kg/min – millilitres per kilogram body weight per minute; Kcal – kilocalories.

## 8.2 Dietary intake

Participants were asked to maintain their habitual dietary intakes over the course of the study. Comparing the baseline food log and the post-training food log, there were no significant differences between groups or time points for absolute intake of energy, measured in kilocalories. There were also no significant differences for macronutrient intake between groups or time points (Table 4). These results do not include the exercise beverage administered during the course of training. The DEI and DEA groups were consuming an additional 270kcal, 39g carbohydrate and 27g protein on training days, whereas the CEI and CEA groups were consuming an addition 240kcal and 60g carbohydrate on training days. When participant intake was analyzed with the beverage added to the post-training food log, there were still no significant differences in total energy intake between groups or time points, and no significant differences between energy intake from macronutrients.

Table 4. Change in energy and macronutrient intake over training intervention.

| Dietary Intake<br>per day | Dairy           |                 | CHO             |                 |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
|                           | Immediate (n=7) | Alternate (n=5) | Immediate (n=7) | Alternate (n=4) |
| Kcal                      | -915 $\pm$ 446  | 408 $\pm$ 116   | 64 $\pm$ 371    | -476 $\pm$ 327  |
| CHO (Kcal)                | -555 $\pm$ 250  | 476 $\pm$ 191   | 73 $\pm$ 202    | 0 $\pm$ 107     |
| CHO (g)                   | -139 $\pm$ 62   | 119 $\pm$ 48    | 18 $\pm$ 51     | -1 $\pm$ 27     |
| PRO (Kcal)                | -120 $\pm$ 64   | 50 $\pm$ 32     | -99 $\pm$ 83    | -73 $\pm$ 42    |
| PRO (g)                   | -30 $\pm$ 16    | 13 $\pm$ 8      | -25 $\pm$ 21    | -18 $\pm$ 10    |
| FAT (Kcal)                | -143 $\pm$ 116  | 79 $\pm$ 144    | 1 $\pm$ 170     | -447 $\pm$ 355  |
| FAT (g)                   | -16 $\pm$ 13    | 9 $\pm$ 16      | 0 $\pm$ 19      | -50 $\pm$ 39    |

Abbreviations: Kcal: kilocalorie; CHO: carbohydrate intake; PRO: protein intake; FAT: fat intake; g - grams. No significant differences between groups or time-points.

Dietary intake of the micronutrients vitamin D and calcium were also analyzed. Participants were ingesting an average of 140 IUs of vitamin D in their diets per day (DEI:  $93.7 \pm 17.9$  pre,  $171.7 \pm 76.8$  post; DEA:  $103.7 \pm 36.2$  pre,  $125.6 \pm 38.3$  post; CEI:  $123.8 \pm 27.0$  pre,  $172.0 \pm 38.9$  post; CEA:  $166.2 \pm 17.2$  pre,  $155.7 \pm 35.6$  post) – there were no significant differences between groups or time points for this measure. However, this does not include the approximate 300 IU/day of vitamin D the DEI and DEA groups were consuming with their beverage [105]. When the fortified vitamin D in the dairy beverages was included in the statistical analyses for intake, there was a significant interaction between group by beverage type and time point. Post-hoc analyses indicated that the dairy beverage groups had significantly higher vitamin D intakes per day post-intervention than the carbohydrate groups at both time points ( $p < 0.001$ ) and that the vitamin D intake of the milk groups was significantly higher post-intervention than at baseline ( $p < 0.001$ ). Despite the significant changes in vitamin D intake for the DEI and DEA groups, serum-25-(OH) D3 status remained unchanged for the different groups and time points, although there was a trend toward significance for the CEA group to have lower 25-(OH) D3 levels overall than any other group ( $p = 0.07$ ). However, this trend could not be attributed to the intervention, and it should be noted that the CEA group had the smallest n (DEI:  $n = 7$ , DEA:  $n = 5$ , CEI:  $n = 6$ , CEA:  $n = 4$ ). When the deltas were analyzed, there was no significant difference between groups (Figure 4).

Mean levels of serum-vitamin D for all groups fell within the reference interval of 20-140nmol/L. However, mean values tended to be at the lower end of this interval, falling between the categories of ‘very deficient’ (<30nmol/L) and ‘insufficient’ (30-75nmol/L). Intra and inter coefficient of variances (CVs) were determined for our vitamin

D measures. Intra CVs for vitamin D were 3.6% (Plate 1) and 6.2% (Plate 2), while the inter-plate CV was 4.9%.

The habitual baseline dietary calcium intake was also not significantly different between groups or time points. When the additional calcium intake from the milk beverages was taken into account, there was a significant interaction between group by beverage type and time point. Post-hoc analyses indicated that the dairy beverage groups had significantly higher calcium intakes per day than the carbohydrate groups at both time points ( $p < 0.05$ ) and that the calcium intake of the milk groups was significantly higher post-intervention than at baseline ( $p < 0.001$ ).

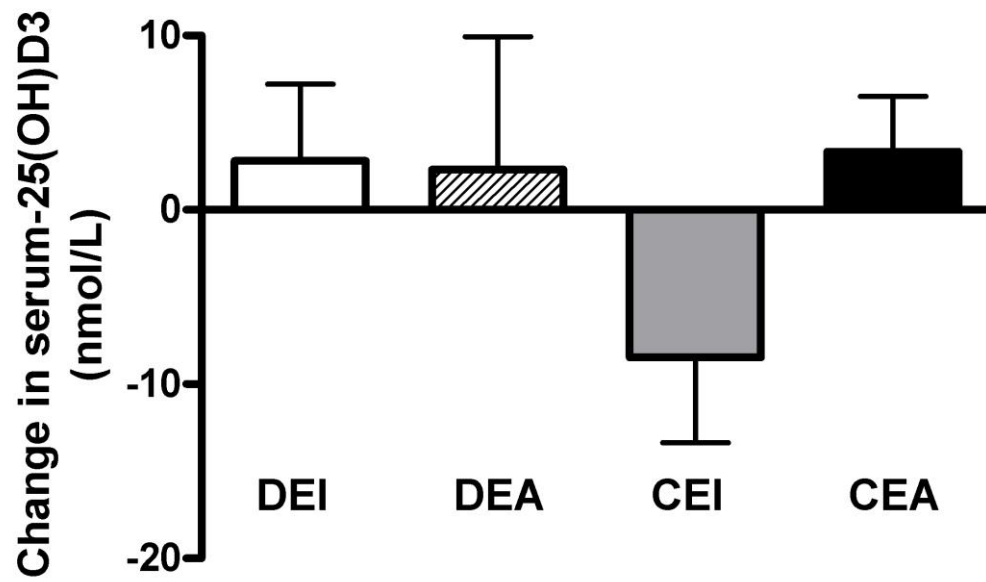


Figure 4. Change in serum-25-(OH)D3 by group.

Abbreviations: DEI – Dairy-Exercise-Immediate group; DEA – Dairy-Exercise-Alternate group; CEI – Carbohydrate-Exercise-Immediate group; CEA – Carbohydrate-Exercise-Alternate group. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. No significant differences between groups or time-points.

### 8.3 Endurance exercise capacity markers

Following completion of the training, there was no significant difference in endurance capacity between groups or time points as measured by maximal heart rate. In contrast, there was a significant main effect for relative  $\text{VO}_{2\text{peak}}$  (ml/kg/min),  $\text{VO}_{2\text{peak}}$  relative to lean mass (ml/kg LM/min) and absolute  $\text{VO}_{2\text{peak}}$  (ml/min) by supplement timing, such that the groups ingesting the beverage immediately post-training had a higher overall  $\text{VO}_{2\text{peak}}$  than those ingesting the beverage at an alternate time. This main effect was seen when we analyzed pre- and post-intervention values, rather than the delta values, and cannot be attributed to the intervention as it reflects subject characteristics rather than change over time. As expected, there was also a main effect for relative  $\text{VO}_{2\text{peak}}$  by time point, such that all groups exhibited an increase in relative  $\text{VO}_{2\text{peak}}$  post-intervention when compared to baseline ( $p < 0.01$ ) (see Figure 5B). When the delta values for relative  $\text{VO}_{2\text{peak}}$  post-intervention were compared to baseline, there were no significant differences between the groups; all groups increased by similar amounts. The main effect for time was not apparent when considering absolute  $\text{VO}_{2\text{peak}}$  or  $\text{VO}_{2\text{peak}}$  relative to lean mass values (Figure 5C), although the change from baseline did exhibit a notable trend for absolute  $\text{VO}_{2\text{peak}}$  ( $p = 0.07$ ) (Figure 5A).

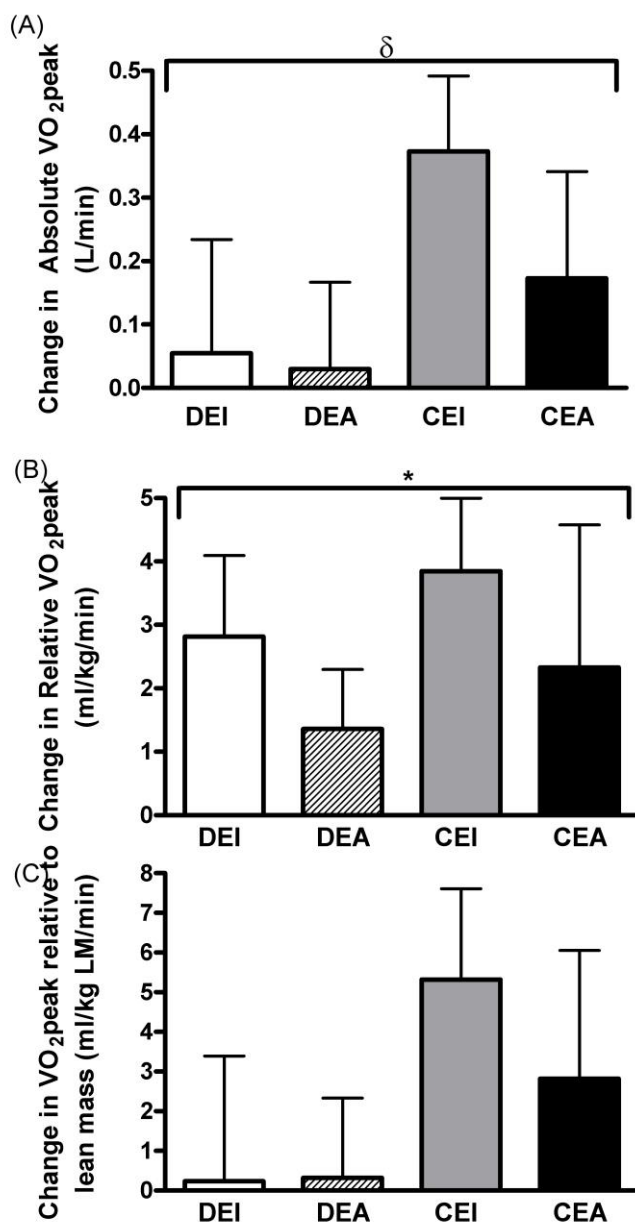


Figure 5. Influence of beverage type and intake timing on change in  $VO_{2peak}$  measures. Absolute  $VO_{2peak}$  (A), relative  $VO_{2peak}$  (B) and relative  $VO_{2peak}$  to lean mass (C).

\* Main effect for increase in relative  $VO_{2peak}$  from baseline ( $p < 0.05$ ).  $\delta$  Trend toward significance for increase in absolute  $VO_{2peak}$  from baseline ( $p = 0.07$ ). DEI:  $n = 7$ . DEA:  $n = 5$ . CEI:  $n = 6$ . CEA:  $n = 4$ . Abbreviations: DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate; L/min – litres per minute; ml/kg/min – millilitres per kilogram per minute; ml/kg LM/min – millilitres per kilogram of lean mass per minute



#### **8.4 Body composition**

There were no significant differences in changes in body composition or fat mass between groups (see Figure 6A and B). However, a main effect for time in regards to lean mass was observed – all groups had a similar significant increase in lean mass following the training ( $p < 0.05$ ) (see Figure 6C). When delta values were analyzed, no significant differences were noted, indicating that all groups increased lean mass by approximately the same amount.

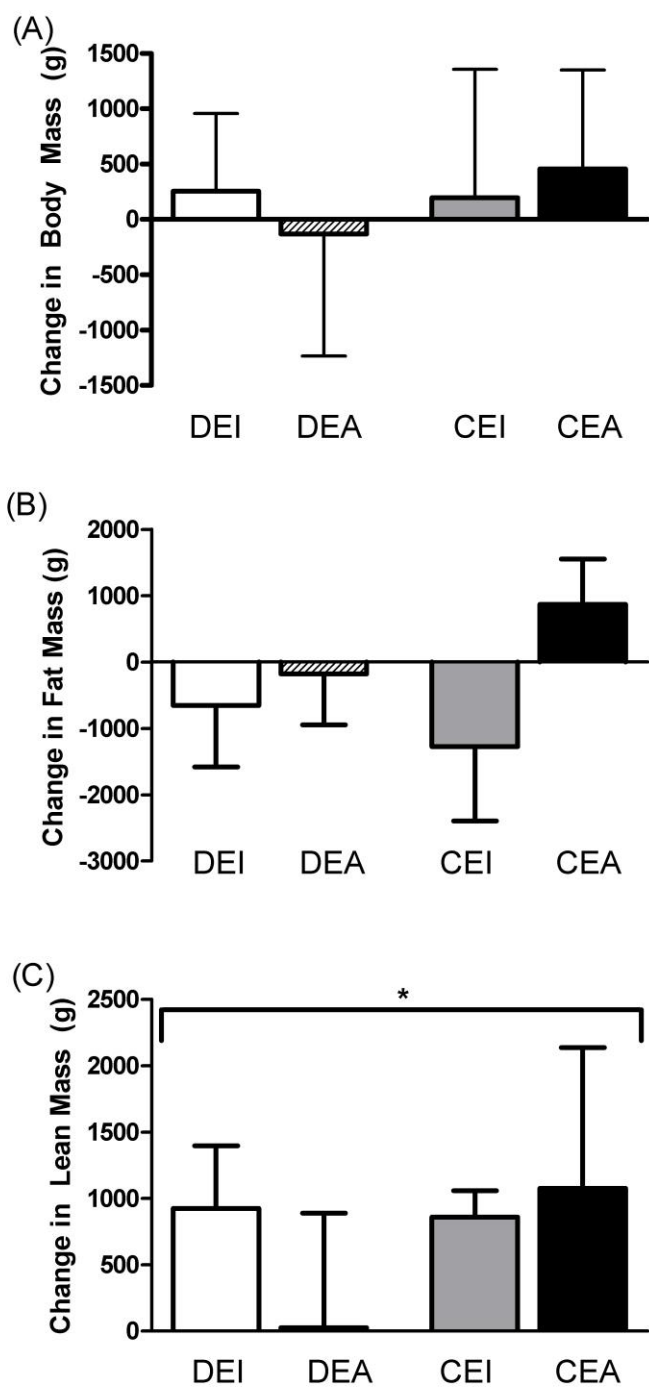


Figure 6. Influence of beverage type and intake timing on changes in body composition. Body mass (A), fat mass (B), and lean mass (C).

\* Main effect for increase in lean mass values from baseline ( $p < 0.05$ ). DEI:  $n = 7$ . DEA:  $n = 5$ . CEI:  $n = 6$ . CEA:  $n = 4$ . Abbreviations: DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate; g – grams.

## 8.5 Blood lipids

A significant main effect for group was observed for total serum cholesterol levels – the DEI and CEI groups had lower total serum cholesterol concentrations overall than the DEA and CEA groups ( $p < 0.05$ ) irrespective of time; however, this difference could not be attributed to the intervention. A similar main effect was also seen for serum LDL cholesterol; again, this difference could not be attributed to the intervention. Despite the main effects for serum total cholesterol and LDL cholesterol, no significant differences for any other blood lipid measures were observed (see Table 5). When delta values were analyzed, no differences between groups were noted for total cholesterol (Figure 7) or any other blood lipid measures. Mean values for the DEI, DEA and CEI groups fell in the desirable ranges for total cholesterol ( $< 5.2 \text{ mmol/L}$ ) and triglycerides ( $< 1.7 \text{ mmol/L}$ ) at both baseline and post-intervention. The CEA group fell outside these ranges at both time points, although it should be noted that this group was both the smallest ( $n = 4$ ) and had the highest average age. At both time points, all groups exhibited HDL values that were lower than the desirable range of  $\geq 1.3 \text{ mmol/L}$ . The DEI, DEA, and CEI groups had LDL values that were higher than the desirable range, but still within the range of ‘low risk’ classification ( $< 3.4 \text{ mmol/L}$ ) at baseline and post-intervention. However, the CEA group had LDL values that fell just above the ‘low risk’ range (baseline:  $3.5 \text{ mmol/L}$ ; post-intervention:  $3.6 \text{ mmol/L}$ ).

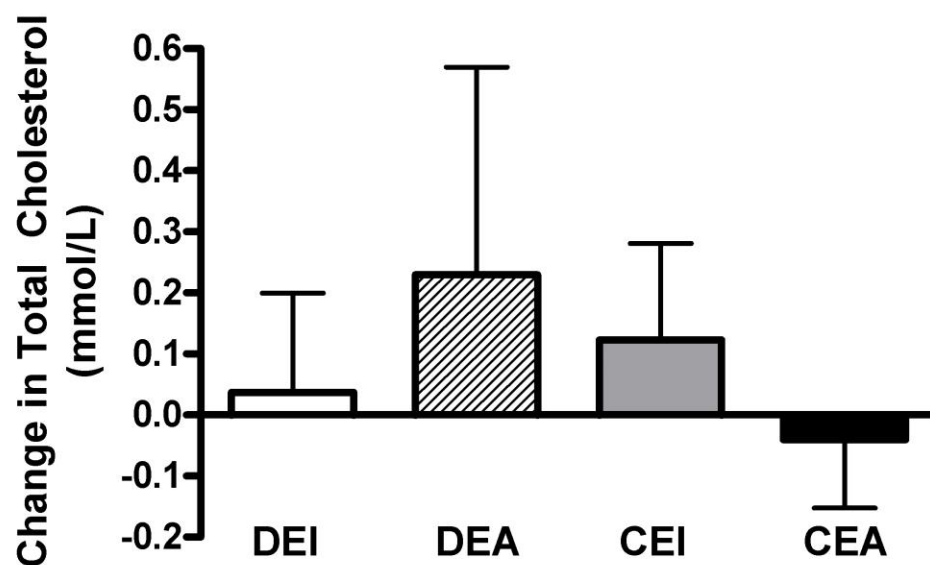


Figure 7. Influence of beverage type and intake timing on change in total blood cholesterol concentration.

DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. Abbreviations: DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate; mmol/L – millimoles per litre

Table 5. Influence of beverage type and intake timing on change in serum lipids.

| Marker<br>(mmol/L) | Dairy            |                 | CHO              |                 |
|--------------------|------------------|-----------------|------------------|-----------------|
|                    | Immediate (n=7)* | Alternate (n=5) | Immediate (n=7)* | Alternate (n=4) |
| TCHOL              | 0.04 ± 0.16      | 0.23 ± 0.34     | 0.12 ± 0.16      | -0.04 ± 0.11    |
| LDL                | 0.02 ± 0.18      | 0.20 ± 0.28     | 0.11 ± 0.10      | -0.05 ± 0.11    |
| TGs                | -0.34 ± 0.45     | -0.04 ± 0.22    | -0.06 ± 0.14     | 0.38 ± 0.62     |
| HDL                | 0.01 ± 0.04      | -0.01 ± 0.05    | 0.04 ± 0.07      | 0.04 ± 0.06     |

Abbreviations: TCHOL: Total cholesterol; LDL: Low-density lipoprotein cholesterol; TGs: Triglycerides; HDL: High-density lipoprotein cholesterol. \*TCHOL and LDL values were significantly lower overall for DEI and CEI groups in comparison to DEA and CEA groups ( $p < 0.05$ ); this finding is not attributable to the intervention. No other significant effects noted.

## 8.6 Inflammatory markers

In regards to the serum inflammatory markers TNF- $\alpha$  (Figure 8A) and CRP (Figure 8B), no significant differences were observed between the experimental groups or at the different time points. Analyses of delta values (post-test value minus pre-test value) confirmed this result. Interestingly, serum concentrations of IL-6 were below the level of detection for the method used for all except for two of the participants, and as such no statistical analysis of IL-6 could be performed. For the anti-inflammatory serum marker adiponectin (Figure 8C), there was a main effect for time, such that all groups demonstrated a decrease in serum adiponectin following the intervention ( $p < 0.05$ ). Statistical analysis of delta values for this marker indicated that each group experienced a statistically similar decrease in adiponectin level.

At both baseline and post-intervention, mean levels for both CRP and TNF- $\alpha$  were within healthy ranges ( $<10\text{mg/L}$  and  $<11.2\text{pg/ml}$  respectively). Mean adiponectin levels were higher than normal ( $3.7\text{-}5.7\mu\text{m/ml}$ ) for all groups at both time points. Coefficient of variances (CVs) were determined within each plate and between plates. For TNF- $\alpha$  we achieved CVs of 2.2% (Plate 1) and 16.2% (Plate 2), for an inter-plate CV of 9.2%. For CRP we achieved CVs of 5.8% (Plate 1) and 8.2% (Plate 2), for an inter-plate CV of 6.6%. For adiponectin we achieved CVs of 7.5% (Plate 1) and 7.4% (Plate 2), for an inter-plate CV of 7.4%.

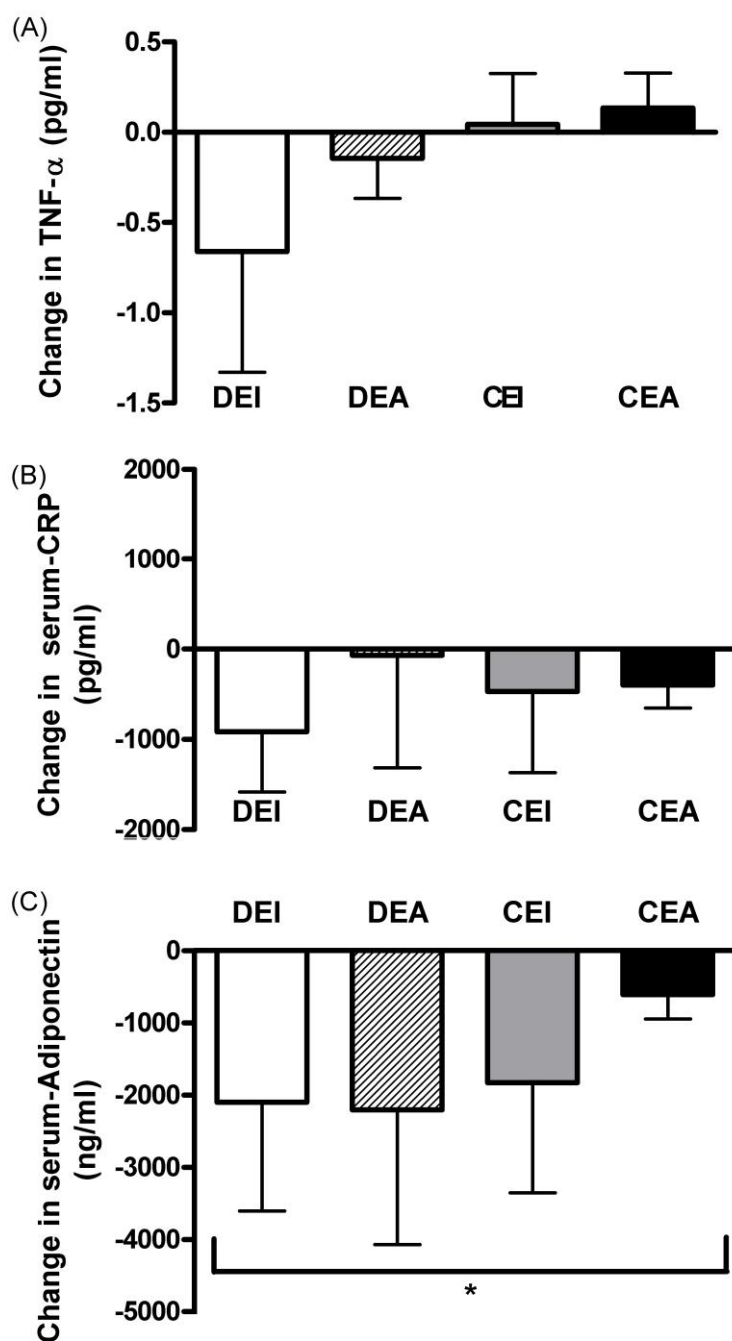


Figure 8. Influence of beverage type and intake timing on change in inflammatory markers. Inflammatory markers TNF- $\alpha$  (A) and CRP (B), and anti-inflammatory marker adiponectin (C).

\* Main effect for reduction in adiponectin levels from baseline ( $p < 0.05$ ). DEI:  $n = 7$ . DEA:  $n = 5$ . CEI:  $n = 6$ . CEA:  $n = 4$ . Abbreviations: TNF- $\alpha$ : Tumor-necrosis-factor-alpha; CRP: C - reactive protein; pg/ml – picograms per millilitre; ng/ml – nanograms per millilitre.

## 8.7 Metabolic markers

There were no significant differences between groups for glucagon or insulin. Similarly, there were no significant changes in leptin levels between groups. Refer to Table 6 for delta values of these markers. As there were no significant interactions for either resting glucose or resting insulin levels, fasting glucose values were determined and the homeostasis model assessment (HOMA) index was calculated for each participant as an estimate of insulin resistance. There were no significant differences between groups or time points for the HOMA index. However, most groups had a trend for lower HOMA index value post-intervention than at baseline ( $p = 0.08$ ). Furthermore, no groups had HOMA index values that were around the insulin-resistance cut-off point of approximately 3 [104].

At both baseline and post-intervention the DEI, CEI and CEA groups had mean values of glucagon that fell within the reference range ( $<60\text{pg/ml}$ ), while the DEA group had mean glucagon values that were higher than this range ( $84.5\text{pg/ml}$  at baseline,  $64.8\text{pg/ml}$  post-intervention). Mean insulin values for all groups fell within the reference range for insulin ( $3\text{-}25\mu\text{IU/ml}$ ) at both time points, as did leptin values ( $0.6\text{-}36.4\text{ng/ml}$ ). Coefficient of variance (CV) was determined for each plate and compared within measures. For glucagon we achieved CVs of 5.9% (Plate 1) and 14.2% (Plate 2), for an inter-plate CV of 10.1%. For leptin we achieved CVs of 4.7% (Plate 1) and 4.3% (Plate 2), for an inter-plate CV of 4.5%. For insulin we achieved CVs of 2.0% (Plate 1) and 8.5% (Plate 2), for an inter-plate CV of 5.3%. For glucose we achieved CVs of 3.8% (Plate 1) and 1.0% (Plate 2), for an inter-plate CV of 2.4%.



Table 6. Influence of beverage type and intake timing on change in fasting serum metabolic markers.

| Marker           | Dairy           |                 | CHO             |                 |
|------------------|-----------------|-----------------|-----------------|-----------------|
|                  | Immediate (n=7) | Alternate (n=5) | Immediate (n=7) | Alternate (n=4) |
| Leptin (ng/ml)   | 0.50 ± 0.85     | 0.08 ± 2.00     | -1.92 ± 1.86    | 1.10 ± 1.07     |
| Glucagon (pg/ml) | 1.34 ± 2.73     | -19.70 ± 8.12   | -0.05 ± 18.17   | -4.61 ± 7.84    |
| Insulin (pg/ml)  | 20 ± 60         | -81 ± 93        | -94 ± 95        | 110 ± 101       |
| HOMA index       | 0.16 ± 0.23     | -0.31 ± 0.40    | -0.32 ± 0.36    | -0.69 ± 0.58    |

DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. No significant differences between groups or time points. Trend toward significance for HOMA index to be lower post-intervention ( $p < 0.08$ ).

## 9. Discussion

The primary purpose of this study was to determine the combined effects of increased physical activity and dairy consumption on body composition,  $\text{VO}_2\text{peak}$ , blood lipids and metabolic markers. The main hypothesis was that combining increased LFM intake with endurance exercise training would augment the physiological and health-related adaptations that would be experienced with training alone; however, the majority of findings of this study do not support this hypothesis. Also, the hypothesis that all groups would experience an increase in  $\text{VO}_2\text{peak}$  was not fully supported. All experimental groups did exhibit a significant increase in relative  $\text{VO}_2\text{peak}$  which was similar between the different groups (Time 1: 41.6ml/min/kg; Time 2: 44.2ml/min/kg;  $p < 0.05$ ). Despite the increases in relative  $\text{VO}_2\text{peak}$ , there were no significant differences in absolute  $\text{VO}_2\text{peak}$  values, although the changes from baseline exhibit a notable trend ( $p = 0.07$ ). Furthermore, the hypothesis that combining training and LFM intake would reduce whole body inflammation and inflammatory markers was also not supported, as there were no significant differences between experimental groups or time points for any inflammatory or anti-inflammatory markers. The combination of endurance training and LFM also did not appear to have any effects on fasting serum cholesterol levels, glucose or insulin.

### 9.1 Vitamin D – intake and serum levels

A surprising finding in this study was that vitamin D (25-(OH) D<sub>3</sub>) status did not change significantly from baseline in any of the groups that consumed LFM. Due to fortification of milk with vitamin D<sub>2</sub> in Canada, we had expected to see an increase or at least maintenance of serum-vitamin D status. It is possible that our observations were due

to a number of different reasons. Firstly, seasonal variations in exposure to ultraviolet radiation may have contributed to variability in results, as the majority of our participants completed the study during the fall and winter months. Furthermore, participants were not taking any other supplements, and as such feeding them 3 servings of low fat milk does not appear to be adequate to maintain serum 25-(OH)D levels. Finally, there is some evidence to suggest that it may take at least 3 months of increased vitamin D intake to see notable changes in serum vitamin D levels [106], and we may have seen no changes with our intervention as it was just barely at the 3-month mark. While 25-(OH)D (a.k.a calcidiol) is not the biologically active form of vitamin D, it does indicate the amount available for transition to the biologically active form, 1,25-(OH)D (a.k.a calcitriol). Interestingly, calcitriol uptake has been shown to be suppressed by calcium ingestion. Zemel et al. saw significant decreases in calcitriol concentrations with dairy supplementation [37]. However, Caron-Jobin et al. found that women who ingested more than 2 servings of dairy per day had higher serum-25(OH)D than their peers ingesting less than 2 servings per day [13]. In addition, they found that the season/time of year of data collection did not affect the relationship between vitamin D and any other anthropometric measures [13]. It should also be noted that there is some controversy as to the most effective method for assessment of serum vitamin D. Our analysis employed the use of ELISA kits, whereas other studies have used radioimmunoassay [13, 37] or high pressure liquid chromatography [107, 108]. It is possible that the method employed in this study underestimated the levels of serum vitamin D, as the results given by the ELISA were at the low end of the reference interval for males 18 years or older, and all of the group averages were well below the population averages for overweight individuals

and individuals not consuming 1 serving of milk per day [109]. However, we see from the CV values that both our intra- and inter-assay CV values are quite low (intra-assay: 3.6%, 6.2%; inter-assay: 4.9%) and so while our results may be low, they are reliable based on these values.

## **9.2 Body composition**

One of the primary expected outcomes of this study was to see a positive shift in body composition. We expected to see a shift in the fat-mass to lean-mass ratio, such that participants would decrease fat-mass and overall body-weight. While we did not observe any difference in body weight or fat mass change, there was a significant increase in lean mass as a result of the intervention. There are a few factors that may have affected these results, such as the addition of 3 servings of LFM, with the many potential bioactive components that milk contains including calcium, and daily endurance exercise.

### *9.2.1 Calcium/dairy intake, and exercise for body composition*

As previously stated, we did not observe any differences between groups or time-points for body weight or fat mass. Additionally, we did not observe any significant differences in calcium intake between the groups at baseline, but when the added dairy intake was taken into account in the DEI and DEA groups, these groups exhibited significantly higher calcium intakes compared to baseline and the CEI and CEA groups. Caron-Jobin et al. also found that individuals ingesting 2 or more servings of dairy per day had increased calcium intake and that this increased intake was significantly related to smaller visceral fat deposits compared to lower dairy and calcium intake [13]. Other studies have looked at milk intake as a whole food, rather than isolating the intake of

calcium, and investigated changes in body weight. Barba et al. noted that children who ingested 2 or more servings of dairy per day had lower body weight than their peers [12]. Again, this is somewhat in contrast to our findings, as there were no significant differences between our groups with the ingestion of 3 additional servings of dairy daily and therefore additional calcium. These discrepancies in findings may be related to the duration of dairy intake – both Barba et al. and Caron-Jobin et al. employed a cross-sectional study design to investigate individuals' habitual dairy intake over the course of many years, and compared them to those who had low habitual dairy intakes over the same period of time [12, 13]. Based on these observations, it is possible that a longer duration of increased dairy intake in the current study may have resulted in similar findings to these two previously reported studies. However, Harvey-Berino et al. did not see any differences in body weight or fat mass change between groups consuming two or three servings of dairy per day compared to those consuming less than 2 servings per day over the course of 12 months, even with daily exercise of ~200 kcal expenditure [56]. Based on these observations, it appears that habitually elevated intake of dairy over the course of years, not just months, may be required in order to provide body composition benefits with endurance training. However, when considering dairy intake and resistance exercise training, a relatively short period of training and intake has been shown to result in fat mass loss, lean mass gain, and overall improvement in body composition [77, 97-100]. It should be noted that the participant populations in the current study was one with elevated BMI and a wide age range, while the previous studies investigating the combined effects of dairy and resistance training recruited healthy young university aged individuals.

It was somewhat surprising that the exercise component of this intervention alone, without dairy intake, did not result in any significant reductions in body mass or fat mass. However, Hulver et al. also found no changes in body mass or fat mass in sedentary, overweight participants after a 6-month low-intensity endurance-training program [110]. Similarly, Stuart et al. observed that a 8-week progressive cycling protocol elicited no changes in lean or fat mass for sedentary participants [111]. In contrast, Kraus et al. saw a significant decrease in body weight in sedentary, overweight participants with a 6-month moderate-intensity endurance-training program [51]. It is possible based on these various observations that the intensity of training is a critical factor and likely contributes to the variability in the results in these different studies. It is important to note that although the studies with similar populations and exercise interventions to our study did not see fat mass change they did note other health benefits, in particular insulin action and sensitivity improvements. These results indicate that the health benefits of exercise are not necessarily weight-dependent.

### 9.2.2 *Lean mass gain*

Interestingly, we did observe the expected increase in lean mass. Most studies that have investigated the effects of endurance training have only report changes in body weight and fat mass, rather than lean mass, and these studies recruit both sedentary and recreationally active participants [51, 61, 110]. However, a resistance training study that included supplementation with the milk proteins casein and whey in one of the experimental groups, observed the greatest increases in lean mass and fat-free mass in the casein and whey group as compared to the control and carbohydrate supplemented groups [112]; it should be noted that the participants involved in this study were already

resistance-trained. The increase in lean mass observed in the current study was consistent across all of our groups, and thus cannot be attributed to the additional intake of LFM. It appears that the exercise protocol itself led to the increase in lean mass in the current study, despite the differing results observed by Stuart et al. and the similar training protocol that was used [111]. In addition, our number of participants and participants BMI's were similar to those involved in the study by Stuart et al. (Stuart et al.: N = 18, BMI = 28-35; this study: N = 22, BMI = 23-35) [111].

### **9.3 VO<sub>2</sub>peak**

The training protocol used in the current study did lead to a significant increase in relative VO<sub>2</sub>peak across all groups following training; however, this finding did not carried over to measures of absolute VO<sub>2</sub>peak or VO<sub>2</sub>peak relative to lean mass. Similarly, Carter et al. found that a 7-week cycling protocol resulted in significant relative VO<sub>2</sub>peak improvements in both men and women [61], and Stuart et al. found the same with an 8-week cycling protocol in sedentary and overweight participants [111]. Neither the study by Carter et al. nor the one by Stuart et al. included beverage supplementation. Therefore, while we cannot attribute any of the VO<sub>2</sub>peak improvements to the beverage type, it is clear from our results and the results of other endurance training studies that cycling, even at low intensities, is beneficial for improving exercise capacity, as measured by relative VO<sub>2</sub>peak.

### **9.4 Blood lipid status**

It was hypothesized that both the endurance training and the increased LFM intake would lead to improved blood lipid status in our participants. However, the results

indicated that neither endurance exercise nor beverage type had any significant post-training effects on blood lipids. Interestingly, Zemel et al. did not find any effect of an additional 3 servings of low fat milk intake, over the course of 28 days, on total cholesterol, HDL cholesterol or triglyceride levels in overweight and obese adults [37], supporting our observations. In contrast, Lorenzen et al. found that high dairy calcium, ingested as 3 servings of milk per day as well as supplementation over 10 days, offset the negative effects of dairy fat to result in overall attenuation of total cholesterol and LDL production [55]. In addition, Caron-Jobin et al. found that women with higher calcium and vitamin D intakes, due to habitual ingestion of 2 or more servings of dairy per day, had lower triglyceride levels and lower total cholesterol concentration [13]. Steinmetz et al. saw a trend for lower total cholesterol, LDL, triglycerides and even HDL with 6 weeks of increased skim milk consumption (approximately 2 servings per day) in college-aged recreationally active men [60]. Ditscheid et al. also found that increased calcium reduced total cholesterol and LDL levels over the course of 4 weeks in young, healthy adults; however, calcium was not dairy-based in their experimental trials [53]. Collectively, these various studies indicate that dairy and calcium ingestion may lead to improvements in cholesterol status. The contrast in findings between these studies and ours is likely due to two factors: how much calcium was ingested daily and when the dairy and/or calcium were ingested. Two of the studies that found increased calcium supplementation improved blood-lipid status were using ingestion levels of 1060mg per day [53] and nearly 2000mg per day [55] whereas our participants were ingesting approximately 900mg of calcium per day. It has been shown that calcium acts within the intestines to bind with fat, creating indigestible soaps which in turn leads to increase fat excretion and



improvements in blood-lipid status [19, 70]. However, in order for this interaction to occur, calcium must be ingested at approximately the same time as the fat. Each of the studies by Ditscheid et al., Lorenzen et al., and Steinmetz et al. involved dietary interventions which required their participants to ingest the dairy and/or calcium at the same time as their regular meals [53, 55, 60]. In contrast, our participants in the DEI group were ingesting their dairy beverage at a time when no other food was ingested, and although we cannot comment with confidence about the circumstances in which the DEA beverages were ingested, it is unlikely that other food was taken at the same time as 6 hours post-intervention often took the participants into the late evening/early morning hours. Furthermore, there were no significant changes in participant reported dietary fat intake post-training, indicating that the lack of serum-lipid improvement was likely not related to increased fat ingestion offsetting any dairy-related benefits.

Considering the effects of exercise, Kraus et al. did not see any change in total cholesterol or LDL for sedentary, overweight participants after a 6-month moderate-intensity endurance-training program [51], similar to what was used in the current study. Interestingly, changes in blood lipids have been observed in overweight and obese individuals with higher training intensities – about 2 miles per day at 75%  $\text{VO}_{2\text{peak}}$  - over the course of 6 months [51]. Therefore, it is possible that changes in blood lipids may occur with a longer period of lower intensity training, or at higher exercise intensities. It is possible that our intervention of 12-weeks of low-intensity cycling needed to be augmented in order to see changes in blood-lipids. We may have seen the expected changes with either higher exercise intensity or longer study duration at the given intensity. An increase in duration is the more likely solution as increasing the

intensity of training may be more problematic, due to many of the participants who were not accustomed to exercise, and dropped out of the current protocol because they found the training challenging, despite the low intensity level.

### **9.5 Inflammatory and anti-inflammatory markers**

This study focussed on three markers of inflammatory status, CRP, TNF- $\alpha$  and IL-6, and one anti-inflammatory marker, adiponectin. Of the inflammatory markers measured, only CRP and TNF- $\alpha$  could be analyzed as IL-6 levels were below the level of detection of the Magpix® system at both baseline and post-testing. While we were able to reliably measure CRP and TNF- $\alpha$ , there were no significant differences in the resting values of either of these markers between groups or over time. As previously stated, the mean values observed for these adipokines were well within the reference ranges both at baseline and after the intervention (CRP reference range: <10mg/L; TNF- $\alpha$ : 0.0–32.5pg/ml), indicating that our results are valid as well as reliable. Surprisingly, the anti-inflammatory marker adiponectin significantly decreased post-intervention compared to baseline ( $p < 0.05$ ). These results would indicate no change in inflammatory status, or perhaps a slight increase in inflammation as adipose-related inflammation has been shown in conjunction with lower adiponectin levels [7, 35, 39, 46]. However, it is unlikely that the decrease in adiponectin indicates increased inflammation, as at both baseline and post-intervention mean adiponectin values were above the healthy reference range of 3.7-5.7 $\mu$ g/ml, even with the post-intervention level decrease. One possible explanation is that the observed decrease was due to improved signalling and efficacy of adiponectin within the body – as the participants become more receptive to the effects of adiponectin, less is secreted and necessary to achieve the same effect [113].

### *9.5.1 Dairy intake and inflammation*

We did not observe any changes in the pro-inflammatory adipokines TNF- $\alpha$  or CRP, and we observed a significant decrease in the anti-inflammatory adipokine adiponectin. In contrast to our findings, Zemel et al. found that dairy consumption (3 servings per day, non-fat milk and/or yoghurt, for 6 months [36] and 1 month [37]) resulted in significant reductions of CRP, TNF- $\alpha$  and IL-6, as well as significant increases in adiponectin, indicating an overall reduction in whole-body inflammation [36, 37]. Similarly, a 1-year longitudinal study by Nui et al. found a significant correlation between high levels of low-fat dairy intake ( $\geq 2$  servings per day) and high blood-adiponectin levels [114]. It is possible that the results of this study were different from the above studies due to our population – while the participants conformed to the inclusion criteria for ‘overweight’, many of those who completed the protocol were not as ‘unfit’ at the beginning of the study as desired. It should be noted that in the studies by Nui et al. [114] and Zemel et al. [36, 37] adiponectin was measured using ELISAs, whereas we used different methodologies (Milliplex®), possibly contributing to some of the differences observed. Furthermore, whole-body inflammation in our participants was not elevated from the outset, as evidenced by the non-detection of IL-6 as well as TNF- $\alpha$  and CRP levels falling within the normal reference ranges. Bearing this in mind, it is possible that we did not see any significant changes in inflammatory and anti-inflammatory markers due to these markers being low at baseline.

### *9.5.2 Exercise and inflammation*

Regarding the effects of exercise, we did not observe changes in our markers to indicate an improvement in inflammatory status. In contrast, Oh et al. found that a moderate-intensity 12-month exercise training program significantly reduced serum levels of TNF- $\alpha$ , IL-6 and leptin, while significantly increasing serum adiponectin levels [115]. This effect was even greater in participants who experienced weight loss through calorie restriction rather than exercise [115]. Hulver et al. found no change in adiponectin levels after a 6-month low-intensity endurance-training program [110]. Interestingly, Hulver et al. did observe that adiponectin levels were increased following weight loss as a result of gastric bypass surgery [110], indicating that changes in adiponectin levels may be directly associated with weight and/or fat loss rather than interventions that induce weight loss – the findings of Kriketos et al. and Oh et al. also support this theory [115, 116]. However, Kriketos et al. did find that 2-3 acute bouts of exercise did increase adiponectin levels, and the increase was sustained over the course of 10 weeks [116]. Despite the increase, no correlation between weight loss, fat oxidation or insulin sensitivity was noted in this trial [116]. In agreement with our findings, Bruce et al. noted a reduction in adiponectin levels in the blood stream with 8 weeks of endurance exercise training in obese individuals [64]. They noted that adiponectin acts in muscle through a pathway to increase FA oxidation [64]; endurance exercise training also increases FA oxidation within muscle [61, 64]. Due to both endurance training and adiponectin producing this outcome, a reduction in adiponectin as a result of training may appear contradictory. It is possible that habitual endurance exercise cancels out the ‘redundant’ effect of adiponectin in this case, and leads to a decrease in the circulating concentrations of adiponectin. Another possible contributing factor as to why we did not

see any positive changes in adiponectin in the current study was that no changes in body mass were observed despite the 12 weeks of training. Clearly, further work is required to better understand the relationship between diet, training, body weight/composition and circulating concentrations of adiponectin.

## **9.6 Metabolic markers**

There were no changes or differences between groups or time points for the metabolic markers: fasting serum glucose, fasting serum insulin, or fasting leptin levels in this study.

### *9.6.1 Fasting insulin*

Our findings also found no effect of exercise or beverage on fasting insulin levels. However, Bruce et al. did see a reduction in fasted blood-insulin levels with eight weeks of endurance training alone, without dairy intake [64], which is in contrast to the current study. Hulver et al. also saw a significant decrease in fasted insulin levels after overweight participants underwent a 6-month low-intensity endurance-training program [110]. Tong et al. found that feeding rats increased amounts of whey protein (one of the primary dairy proteins) led to significantly lower fasted insulin levels as compared to a group consuming no whey at all; however, this result was seen with a high dietary intake of whey (15% of their dietary kilocalories) [117]. In comparison, our participants were ingesting approximately 2-3% of their dietary kilocalories as whey protein, considering that whey makes up approximately 20% of bovine milk [118]. In conclusion, recent research indicates that endurance exercise is beneficial for improving fasting insulin levels in overweight individuals. This study did not observe this improvement perhaps

due to a participant population that was more ‘fit’ than an average overweight or sedentary individual. Perhaps a more prolonged intervention may also have been necessary to see improvements in insulin status. While this study did not observe any changes due to milk intake, there is support from animal studies indicate that supplementation with whey isolate may be beneficial for insulin status. Based on these different observations, it is apparent that more research in this area is required, especially to better understand the interaction between milk intake in humans and resting insulin levels.

Together, these diet- and exercise-studies indicate that neither dairy nor endurance exercise appear to have any beneficial effect on blood glucose levels. In contrast, endurance exercise does appear to beneficially affect insulin levels, although the effect of milk upon insulin remains unclear.

#### *9.6.2 Fasting glucose and HOMA index*

The current study only looked at fasting insulin levels, and thus we are unable to extrapolate these results to better understand how the participants would respond to a post-prandial glucose load. Perhaps if we had performed an oral glucose tolerance test before and after the intervention, changes in insulin responsiveness would have been observed. However, in order to elaborate on the results, the fasting glucose levels for our participants were determined. As expected based on the literature, neither the exercise protocol, the beverage type, nor intake timing appeared to have any effect on fasting blood glucose in our participants. Lunn et al. also found that milk intake combined with endurance exercise training did not appear to have any effect fasting blood-glucose levels

[88]. Lee et al. did not find beverage type (water, CHO-drink, LFM, LFM+CHO) affected resting blood-glucose levels with endurance training [93]. Similarly, eight weeks of endurance training alone, without dairy intake, has been observed to have little to no effect on fasting blood-glucose [64]. This effect does not appear to be duration dependent, as another study had overweight adults complete a 6-month low-intensity endurance-training program, and no changes in fasted glucose levels were observed [110]. To date, results of studies involving human participants indicate that there is no evidence to suggest that the additional consumption of LFM, with or without exercise, has any effect on fasting glucose levels.

We used the fasting glucose values to calculate the HOMA index. Values for the HOMA index were then analyzed to estimate insulin resistance for individual participants and the different experimental groups. While there were no significant differences seen between groups or time points, there was a trend for the HOMA index to be lower for all groups post-intervention when compared to baseline ( $p < 0.08$ ). Struijk et al. also did not observe any correlation between habitual dairy intake and insulin resistance, as measured by the HOMA index and they did not report any trends [119]. Interestingly, Tong et al. found that when Wistar rats are fed increased amounts of whey protein (one of the primary dairy proteins) significantly lower HOMA index values are observed [117]. As previously stated, the rats were fed a diet that was composed of 15% whey protein, whereas our participants' diets contained 2-3% whey from the dairy beverage. Other studies have observed statistically significant improvements in a calculated insulin sensitivity index in humans with 8-weeks of cycling, although the calculation used was  $[10,000/\text{sq.root of (fasting glucose} \times \text{fasting insulin)}] \times (\text{mean glucose} \times \text{mean insulin})$

during OGTT)] rather than the HOMA index [64]. Longer duration protocols (12 months) have also seen significant improvements in the HOMA index with moderate-intensity endurance exercise training [115]. Interestingly, despite improvements in the HOMA index with training in an overweight/obese population, GLUT-4 expression does not appear to change with 8 weeks of endurance training on a cycle ergometer [111], which suggests improvements in signalling may account for the improvements in the HOMA index within 8 weeks of training in this population. Taking the findings of these various studies and our study together, there is no clear relationship between milk intake and measures of insulin resistance. However, there is good evidence to suggest that training alone likely improves markers of insulin sensitivity.

## **9.7 Summary of findings**

In summary, the results of this study support the hypothesis that 12 weeks of exercise endurance training results in an increase in lean mass in absence of weight loss ( $p < 0.05$ ) – this hypothesis was supported regardless of the type or timing of beverage consumed by participants. Furthermore, a significant increase in relative  $\text{VO}_{2\text{peak}}$  ( $p < 0.05$ ) coupled with a trend toward significance for absolute  $\text{VO}_{2\text{peak}}$  increase ( $p = 0.07$ ) was observed. Surprisingly, there was a significant main effect for the anti-inflammatory marker adiponectin to be lower across all groups at the end of the 12-week exercise intervention ( $p < 0.05$ ). However, the main hypothesis that the groups consuming LFM would exhibit increased benefit from the exercise training was not supported. Furthermore, there were no changes observed in any group for the measures of serum cholesterol and triglycerides, serum glucose and insulin, or serum pro-inflammatory markers. While our measures did not allow us to speculate about how participants would



respond to a glycemic load as an adaptation to the training, we were able to perform a HOMA index calculation which indicated that all groups exhibited a reduction in this index post intervention ( $p = 0.08$ ) which suggests that the 12-week endurance exercise intervention has the potential to reduce insulin resistance in a sedentary population.

Although the primary novel hypotheses of this study were not supported, the significant results indicate that this 12-week cycling intervention was effective for producing a training effect in a sedentary and primarily overweight population. While the training effect was limited to lean mass gain and  $\text{VO}_2\text{peak}$  improvement, this intervention does have the potential for further improved adaptations.

## **9.8 Implications and strengths**

This area of research, investigating the combined interventions of dairy and exercise and their effects on weight management and inflammation, is important as the incidences of overweight and obesity are widespread and concerning. Obesity brings with it risk factors for many other health problems, it is essential for healthy behavioural changes to be integrated into daily life to combat obesity and the associated risk factors. The purpose of our study was to investigate a relatively easy lifestyle strategy that can be readily implemented for overweight individuals. This strategy aimed to address the issue of weight management and also the chronic and elevated inflammation and oxidative stress these individuals experience. A strength of this study was that participation brought otherwise sedentary individuals up to recommended levels of both daily activity (30-60min/d of light-moderate exercise at 50-65%HRmax, ~5d/wk [82, 120] and daily dairy intake (at least 2 servings/day) [69]. We did not observe most of the positive adaptations

that we had hypothesized would be observed in response to the intervention, and this may be partially related to the low-intensity exercise protocol that was used. However, while a higher intensity may have produced more physiologically significant results, it is also likely that our adherence rate would have been much lower, and dropout rate higher considering the sample population and demographic. Furthermore, considering that the intensity of training complied with Health Canada's recommendations for physical activity, it is also possible that the duration of study was too short to elicit the adaptations based on the intensity used, rather than the intensity being too low. While the results observed may not have been expected, they do reflect an intervention that is more appealing and practical for this at-risk population. As such, a more prolonged intervention at the same intensity may be necessary to elicit the expected physiological benefits while being more desirable and plausible for this population.

## **9.9 Limitations and Future Directions**

There were a number of limitations with the current study that warrant further discussion. We were unable to recruit the desired number of participants, only enrolling 40 of the expected 56 participants. It should be noted that of the 40 who began the study, only 23 completed it, resulting in a significant drop-out rate of 42.3%. Furthermore, one of our participants experienced pulmonary illness toward the end of the study – although he completed the training and testing protocols, his post-training results had to be excluded from statistical analysis, leaving a final  $n = 22$ . This necessary exclusion was especially disappointing considering that his individual post-test results, taken during his illness, were comparable to his healthy pre-test results, indicating that a healthy post-test would have resulted in improvement. Based on this  $n$ , power calculations were performed

using an alpha of 0.05 and effect sizes of the main outcomes (ranging from 0.02-0.3) which resulted in a final power of less than 0.01. An a priori power analysis was also performed to determine the required sample size to give a power of 0.8 based on the obtained data, and these indicated that the current study would need a minimum of 238 participants. This would suggest that the current sample was insufficient to elicit the expected outcomes.

Based on informal interviews and discussion with the participants, the significant drop-out rate was related to two issues: the location of daily training and the 12-week training time period. Due to space constraints on the main Brock campus, the training took place at the Brock Research and Innovation Centre, which was not easily accessible for participants who did not have access to a vehicle. Furthermore, the 12-weeks of training had to be split up over two semesters. The change in semesters brought its own set of challenges: class schedules that permitted regular training during the fall semester were not so flexible during the winter semester, and seasonal weather changes made travel to and from the Centre even more difficult and inconvenient. Participants who did not drop out altogether for these reasons missed many days that had to be made up later, pushing the 'end date' of the study over a month longer than anticipated. Furthermore, the researchers had no feasible method other than the 'honour code' to ensure that participants were following the guidelines of the study outside of their hour of training. For example, there was no method of ensuring the DEA and CEA groups consumed their beverage at the appropriate time, or at all, and there was no way to ensure that other lifestyle changes – such as eating habits or intermural sports – were not changed or added to participant routines during the course of the study.

Considering the outcomes of this study, it is difficult to say where to go next as these results are inconclusive. However, as this area of research has the potential for beneficial results, especially for the overweight/inactive population, it would be valuable to repeat this study or to run another training cohort to improve participant numbers and statistical power. If this study is to be repeated with university students, shorter study duration for improved adherence over the course of one semester may be necessary. On the other hand, longer study duration may be necessary to see adaptations in this population at this intensity of exercise, so perhaps a population with a consistent schedule should be considered for recruitment of participants. A similar study may investigate post-prandial glucose and insulin responses alongside fasted measures. In order to better determine changes in inflammatory markers, it may be beneficial to repeat this protocol with participants who suffer from dyslipidemia as well as being overweight. Furthermore, including muscle tissue sampling and analysis would be important for looking directly at oxidative stress markers and antioxidant levels. This would allow for investigation into how the intervention is affecting this health issue, as the current methodology only allows assumptions to be made through inflammatory marker results.

## 10. Conclusion

In summary, the findings of this study do not support the original hypotheses. Based on the current observations, there appears to be no added benefit of milk consumption coupled with 12 weeks of exercise training in comparison to 12 weeks of exercise training alone or a carbohydrate beverage coupled with 12 weeks of exercise, on measures of fat and lean mass, changes in blood lipids, metabolic markers, or whole body inflammation. There were no significant changes in body mass or fat mass, although interestingly lean mass did increase significantly from baseline in all groups, as did exercise capacity, as measured by relative  $\text{VO}_{2\text{peak}}$ . There were no significant changes in markers of inflammation due to increased dairy intake and increased endurance training, and there were no significant changes in blood lipid or metabolic markers. Furthermore, timing of beverage consumption did not appear to have any significance for augmenting adaptations due to training over the course of 12 weeks. Therefore, based on the current study, there does not appear to be added benefits to combining increased low-fat dairy intake with moderate intensity endurance exercise for 12 weeks in a population at risk for become obese.

## 11. References

1. Wonisch, W., A. Falk, I. Sundl, B.M. Winklhofer-Roob, and M. Lindschinger, *Oxidative stress increases continuously with BMI and age with unfavourable profiles in males*. Aging Male, 2012.
2. Calder, P.C., N. Ahluwalia, F. Brouns, T. Buetler, K. Clement, K. Cunningham, K. Esposito, L.S. Jonsson, H. Kolb, M. Lansink, A. Marcos, A. Margioris, N. Matusheski, H. Nordmann, J. O'Brien, G. Pugliese, S. Rizkalla, C. Schalkwijk, J. Tuomilehto, J. Warnberg, B. Watzl, and B.M. Winklhofer-Roob, *Dietary factors and low-grade inflammation in relation to overweight and obesity*. Br J Nutr, 2011. **106 Suppl 3**: p. S5-78.
3. Codoner-Franch, P., V. Valls-Belles, A. Arilla-Codoner, and E. Alonso-Iglesias, *Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress*. Transl Res, 2011. **158**(6): p. 369-84.
4. Snel, M., J.T. Jonker, J. Schoones, H. Lamb, A. de Roos, H. Pijl, J.W. Smit, A.E. Meinders, and I.M. Jazet, *Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions*. Int J Endocrinol, 2012. **2012**: p. 983814.
5. WHO. *Global Database on Body Mass Index*. 2006 [cited 2013 19/03]; Available from: <http://apps.who.int/bmi/>.
6. Vincent, H.K., K.E. Innes, and K.R. Vincent, *Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity*. Diabetes Obes Metab, 2007. **9**(6): p. 813-39.
7. Wang, H., L.M. Steffen, B. Vessby, S. Basu, J. Steinberger, A. Moran, D.R. Jacobs, Jr., C.P. Hong, and A.R. Sinaiko, *Obesity modifies the relations between serum markers of dairy fats and inflammation and oxidative stress among adolescents*. Obesity (Silver Spring), 2011. **19**(12): p. 2404-10.
8. Devries, M.C., I.A. Samjoo, M.J. Hamadeh, and M.A. Tarnopolsky, *Effect of endurance exercise on hepatic lipid content, enzymes, and adiposity in men and women*. Obesity (Silver Spring), 2008. **16**(10): p. 2281-8.
9. Thomas, E.L., G. Frost, S.D. Taylor-Robinson, and J.D. Bell, *Excess body fat in obese and normal-weight subjects*. Nutr Res Rev, 2012. **25**(1): p. 150-61.
10. Duncan, B.B., M.I. Schmidt, L.E. Chambless, A.R. Folsom, M. Carpenter, and G. Heiss, *Fibrinogen, other putative markers of inflammation, and weight gain in middle-aged adults--the ARIC study*. Atherosclerosis Risk in Communities. Obes Res, 2000. **8**(4): p. 279-86.
11. Van Loan, M., *The role of dairy foods and dietary calcium in weight management*. J Am Coll Nutr, 2009. **28 Suppl 1**: p. 120S-9S.
12. Barba, G., E. Troiano, P. Russo, A. Venezia, and A. Siani, *Inverse association between body mass and frequency of milk consumption in children*. Br J Nutr, 2005. **93**(1): p. 15-9.
13. Caron-Jobin, M., A.S. Morisset, A. Tremblay, C. Huot, D. Legare, and A. Tchernof, *Elevated serum 25(OH)D concentrations, vitamin D, and calcium intakes are associated with reduced adipocyte size in women*. Obesity (Silver Spring), 2011. **19**(7): p. 1335-41.
14. Ha, E. and M.B. Zemel, *Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people (review)*. J Nutr Biochem, 2003. **14**(5): p. 251-8.

15. Krissansen, G.W., *Emerging health properties of whey proteins and their clinical implications*. J Am Coll Nutr, 2007. **26**(6): p. 713S-23S.
16. Lorenzen, J.K., S. Nielsen, J.J. Holst, I. Tetens, J.F. Rehfeld, and A. Astrup, *Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake*. Am J Clin Nutr, 2007. **85**(3): p. 678-87.
17. Nicklas, B.J., T. You, and M. Pahor, *Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training*. CMAJ, 2005. **172**(9): p. 1199-209.
18. Rossouw, J.E., E.M. Burger, P. Van der Vyver, and J.J. Ferreira, *The effect of skim milk, yoghurt, and full cream milk on human serum lipids*. Am J Clin Nutr, 1981. **34**(3): p. 351-6.
19. Thomas, A.P., T.N. Dunn, J.B. Drayton, P.J. Oort, and S.H. Adams, *A high calcium diet containing nonfat dry milk reduces weight gain and associated adipose tissue inflammation in diet-induced obese mice when compared to high calcium alone*. Nutr Metab (Lond), 2012. **9**(1): p. 3.
20. Zemel, M.B., *Role of calcium and dairy products in energy partitioning and weight management*. Am J Clin Nutr, 2004. **79**(5): p. 907S-912S.
21. Zemel, M.B., *The role of dairy foods in weight management*. J Am Coll Nutr, 2005. **24**(6 Suppl): p. 537S-46S.
22. Zemel, M.B., *Proposed role of calcium and dairy food components in weight management and metabolic health*. Phys Sportsmed, 2009. **37**(2): p. 29-39.
23. Kelley, G.A., K.S. Kelley, and Z. Vu Tran, *Aerobic exercise, lipids and lipoproteins in overweight and obese adults: a meta-analysis of randomized controlled trials*. Int J Obes (Lond), 2005. **29**(8): p. 881-93.
24. Lakka, T.A. and C. Bouchard, *Physical activity, obesity and cardiovascular diseases*. Handb Exp Pharmacol, 2005(170): p. 137-63.
25. Phillips, S.M., H.J. Green, M.A. Tarnopolsky, G.J. Heigenhauser, and S.M. Grant, *Progressive effect of endurance training on metabolic adaptations in working skeletal muscle*. Am J Physiol, 1996. **270**(2 Pt 1): p. E265-72.
26. Strasser, B., M. Arvandi, and U. Siebert, *Resistance training, visceral obesity and inflammatory response: a review of the evidence*. Obes Rev, 2012.
27. Anderson, A.L., T.B. Harris, F.A. Tylavsky, S.E. Perry, D.K. Houston, J.S. Lee, A.M. Kanaya, and N.R. Sahyoun, *Dietary patterns, insulin sensitivity and inflammation in older adults*. Eur J Clin Nutr, 2012. **66**(1): p. 18-24.
28. Barzilay, J.I., C. Forsberg, S.R. Heckbert, M. Cushman, and A.B. Newman, *The association of markers of inflammation with weight change in older adults: the Cardiovascular Health Study*. Int J Obes (Lond), 2006. **30**(9): p. 1362-7.
29. Bastard, J.P., M. Maachi, C. Lagathu, M.J. Kim, M. Caron, H. Vidal, J. Capeau, and B. Feve, *Recent advances in the relationship between obesity, inflammation, and insulin resistance*. Eur Cytokine Netw, 2006. **17**(1): p. 4-12.
30. Dandona, P., A. Aljada, and A. Bandyopadhyay, *Inflammation: the link between insulin resistance, obesity and diabetes*. Trends Immunol, 2004. **25**(1): p. 4-7.
31. Engstrom, G., B. Hedblad, L. Stavenow, P. Lind, L. Janzon, and F. Lindgarde, *Inflammation-sensitive plasma proteins are associated with future weight gain*. Diabetes, 2003. **52**(8): p. 2097-101.

32. Rosa, F.T., M.A. Zulet, J.S. Marchini, and J.A. Martinez, *Bioactive compounds with effects on inflammation markers in humans*. Int J Food Sci Nutr, 2012.
33. Skinner, A.C., M.J. Steiner, F.W. Henderson, and E.M. Perrin, *Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood*. Pediatrics, 2010. **125**(4): p. e801-9.
34. Stancliffe, R.A., T. Thorpe, and M.B. Zemel, *Dairy attenuates oxidative and inflammatory stress in metabolic syndrome*. Am J Clin Nutr, 2011. **94**(2): p. 422-30.
35. Trayhurn, P. and I.S. Wood, *Adipokines: inflammation and the pleiotropic role of white adipose tissue*. Br J Nutr, 2004. **92**(3): p. 347-55.
36. Zemel, M.B. and X. Sun, *Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans*. J Nutr, 2008. **138**(6): p. 1047-52.
37. Zemel, M.B., X. Sun, T. Sobhani, and B. Wilson, *Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects*. Am J Clin Nutr, 2010. **91**(1): p. 16-22.
38. Henriksen, E.J. and S. Jacob, *Modulation of metabolic control by angiotensin converting enzyme (ACE) inhibition*. J Cell Physiol, 2003. **196**(1): p. 171-9.
39. Golbidi, S., A. Mesdaghinia, and I. Laher, *Exercise in the metabolic syndrome*. Oxid Med Cell Longev, 2012. **2012**: p. 349710.
40. Roberts, C.K. and K.K. Sindhu, *Oxidative stress and metabolic syndrome*. Life Sci, 2009. **84**(21-22): p. 705-12.
41. Shi, H., M.V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J.S. Flier, *TLR4 links innate immunity and fatty acid-induced insulin resistance*. J Clin Invest, 2006. **116**(11): p. 3015-25.
42. Scott, A., K.M. Khan, J.L. Cook, and V. Duronio, *What is "inflammation"? Are we ready to move beyond Celsus?* Br J Sports Med, 2004. **38**(3): p. 248-9.
43. Hui, J.M., A. Hodge, G.C. Farrell, J.G. Kench, A. Kriketos, and J. George, *Beyond insulin resistance in NASH: TNF-alpha or adiponectin?* Hepatology, 2004. **40**(1): p. 46-54.
44. Devries, M.C., M.J. Hamadeh, A.W. Glover, S. Raha, I.A. Samjoo, and M.A. Tarnopolsky, *Endurance training without weight loss lowers systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women*. Free Radic Biol Med, 2008. **45**(4): p. 503-11.
45. Kim, H.J., T. Higashimori, S.Y. Park, H. Choi, J. Dong, Y.J. Kim, H.L. Noh, Y.R. Cho, G. Cline, Y.B. Kim, and J.K. Kim, *Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo*. Diabetes, 2004. **53**(4): p. 1060-7.
46. Chu, S.H., M.K. Lee, K.Y. Ahn, J.A. Im, M.S. Park, D.C. Lee, J.Y. Jeon, and J.W. Lee, *Chemerin and adiponectin contribute reciprocally to metabolic syndrome*. PLoS One, 2012. **7**(4): p. e34710.
47. Wood, I.S., F.P. de Heredia, B. Wang, and P. Trayhurn, *Cellular hypoxia and adipose tissue dysfunction in obesity*. Proc Nutr Soc, 2009. **68**(4): p. 370-7.
48. Das, U.N., *Is obesity an inflammatory condition?* Nutrition, 2001. **17**(11-12): p. 953-66.
49. Fan, J. and T. Watanabe, *Inflammatory reactions in the pathogenesis of atherosclerosis*. J Atheroscler Thromb, 2003. **10**(2): p. 63-71.



50. Booth, F.W. and C.K. Roberts, *Linking performance and chronic disease risk: indices of physical performance are surrogates for health*. Br J Sports Med, 2008. **42**(12): p. 950-2.
51. Kraus, W.E., J.A. Houmard, B.D. Duscha, K.J. Knetzger, M.B. Wharton, J.S. McCartney, C.W. Bales, S. Henes, G.P. Samsa, J.D. Otvos, K.R. Kulkarni, and C.A. Slentz, *Effects of the amount and intensity of exercise on plasma lipoproteins*. N Engl J Med, 2002. **347**(19): p. 1483-92.
52. Bendsen, N.T., A.L. Hother, S.K. Jensen, J.K. Lorenzen, and A. Astrup, *Effect of dairy calcium on fecal fat excretion: a randomized crossover trial*. Int J Obes (Lond), 2008. **32**(12): p. 1816-24.
53. Ditscheid, B., S. Keller, and G. Jahreis, *Cholesterol metabolism is affected by calcium phosphate supplementation in humans*. J Nutr, 2005. **135**(7): p. 1678-82.
54. Jacobsen, R., J.K. Lorenzen, S. Toubro, I. Krog-Mikkelsen, and A. Astrup, *Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion*. Int J Obes (Lond), 2005. **29**(3): p. 292-301.
55. Lorenzen, J.K. and A. Astrup, *Dairy calcium intake modifies responsiveness of fat metabolism and blood lipids to a high-fat diet*. Br J Nutr, 2011: p. 1-10.
56. Harvey-Berino, J., B.C. Gold, R. Lauber, and A. Starinski, *The impact of calcium and dairy product consumption on weight loss*. Obes Res, 2005. **13**(10): p. 1720-6.
57. Thompson, W.G., N. Rostad Holdman, D.J. Janzow, J.M. Slezak, K.L. Morris, and M.B. Zemel, *Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults*. Obes Res, 2005. **13**(8): p. 1344-53.
58. Zemel, M.B., W. Thompson, A. Milstead, K. Morris, and P. Campbell, *Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults*. Obes Res, 2004. **12**(4): p. 582-90.
59. Zemel, M.B., J. Richards, A. Milstead, and P. Campbell, *Effects of calcium and dairy on body composition and weight loss in African-American adults*. Obes Res, 2005. **13**(7): p. 1218-25.
60. Steinmetz, K.A., M.T. Childs, C. Stimson, L.H. Kushi, P.G. McGovern, J.D. Potter, and W.K. Yamanaka, *Effect of consumption of whole milk and skim milk on blood lipid profiles in healthy men*. Am J Clin Nutr, 1994. **59**(3): p. 612-8.
61. Carter, S.L., C. Rennie, and M.A. Tarnopolsky, *Substrate utilization during endurance exercise in men and women after endurance training*. Am J Physiol Endocrinol Metab, 2001. **280**(6): p. E898-907.
62. McKenzie, S., S.M. Phillips, S.L. Carter, S. Lowther, M.J. Gibala, and M.A. Tarnopolsky, *Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans*. Am J Physiol Endocrinol Metab, 2000. **278**(4): p. E580-7.
63. Miyazaki, H., S. Oh-ishi, T. Ookawara, T. Kizaki, K. Toshinai, S. Ha, S. Haga, L.L. Ji, and H. Ohno, *Strenuous endurance training in humans reduces oxidative stress following exhausting exercise*. Eur J Appl Physiol, 2001. **84**(1-2): p. 1-6.
64. Bruce, C.R., A.B. Thrush, V.A. Mertz, V. Bezaire, A. Chabowski, G.J. Heigenhauser, and D.J. Dyck, *Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content*. Am J Physiol Endocrinol Metab, 2006. **291**(1): p. E99-E107.

65. Donges, C.E., R. Duffield, and E.J. Drinkwater, *Effects of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body composition*. Med Sci Sports Exerc, 2010. **42**(2): p. 304-13.
66. Haug, A., A.T. Hostmark, and O.M. Harstad, *Bovine milk in human nutrition--a review*. Lipids Health Dis, 2007. **6**: p. 25.
67. Zemel, M.B., H. Shi, B. Greer, D. Dirienzo, and P.C. Zemel, *Regulation of adiposity by dietary calcium*. FASEB J, 2000. **14**(9): p. 1132-8.
68. Meisel, H., *Biochemical properties of peptides encrypted in bovine milk proteins*. Curr Med Chem, 2005. **12**(16): p. 1905-19.
69. *Dairy Nutrition*. [cited 2013 19/03]; Available from: [www.dairynutrition.ca](http://www.dairynutrition.ca).
70. Boon, N., G.B. Hul, J.H. Stegen, W.E. Sluijsmans, C. Valle, D. Langin, N. Viguerie, and W.H. Saris, *An intervention study of the effects of calcium intake on faecal fat excretion, energy metabolism and adipose tissue mRNA expression of lipid-metabolism related proteins*. Int J Obes (Lond), 2007. **31**(11): p. 1704-12.
71. Sharpe, S.J., G.D. Gamble, and D.N. Sharpe, *Cholesterol-lowering and blood pressure effects of immune milk*. Am J Clin Nutr, 1994. **59**(4): p. 929-34.
72. St-Onge, M.P., E.R. Farnworth, and P.J. Jones, *Consumption of fermented and nonfermented dairy products: effects on cholesterol concentrations and metabolism*. Am J Clin Nutr, 2000. **71**(3): p. 674-81.
73. Holmer-Jensen, J., T. Karhu, L.S. Mortensen, S.B. Pedersen, K.H. Herzig, and K. Hermansen, *Differential effects of dietary protein sources on postprandial low-grade inflammation after a single high fat meal in obese non-diabetic subjects*. Nutr J, 2011. **10**: p. 115.
74. Holick, M.F., *High prevalence of vitamin D inadequacy and implications for health*. Mayo Clin Proc, 2006. **81**(3): p. 353-73.
75. Shi, H., A.W. Norman, W.H. Okamura, A. Sen, and M.B. Zemel, *1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action*. FASEB J, 2001. **15**(14): p. 2751-3.
76. Madureira, A.R., T. Tavares, A.M. Gomes, M.E. Pintado, and F.X. Malcata, *Invited review: physiological properties of bioactive peptides obtained from whey proteins*. J Dairy Sci, 2010. **93**(2): p. 437-55.
77. Roy, B.D., *Milk: the new sports drink? A Review*. J Int Soc Sports Nutr, 2008. **5**: p. 15.
78. Bos, C., C.C. Metges, C. Gaudichon, K.J. Petzke, M.E. Pueyo, C. Morens, J. Everwand, R. Benamouzig, and D. Tome, *Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans*. J Nutr, 2003. **133**(5): p. 1308-15.
79. Maughan, R.J., J.B. Leiper, and G.E. Vist, *Gastric emptying and fluid availability after ingestion of glucose and soy protein hydrolysate solutions in man*. Exp Physiol, 2004. **89**(1): p. 101-8.
80. Pal, S. and V. Ellis, *The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals*. Obesity (Silver Spring), 2010. **18**(7): p. 1354-9.
81. Aziz, M., A. Jacob, A. Matsuda, and P. Wang, *Review: milk fat globule-EGF factor 8 expression, function and plausible signal transduction in resolving inflammation*. Apoptosis, 2011. **16**(11): p. 1077-86.

82. Garber, C.E., B. Blissmer, M.R. Deschenes, B.A. Franklin, M.J. Lamonte, I.M. Lee, D.C. Nieman, and D.P. Swain, *American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise*. Med Sci Sports Exerc, 2011. **43**(7): p. 1334-59.
83. Durstine, J.L., P.W. Grandjean, C.A. Cox, and P.D. Thompson, *Lipids, lipoproteins, and exercise*. J Cardiopulm Rehabil, 2002. **22**(6): p. 385-98.
84. Thomas, K., P. Morris, and E. Stevenson, *Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks*. Appl Physiol Nutr Metab, 2009. **34**(1): p. 78-82.
85. Neubauer, O., S. Reichhold, A. Nersesyan, D. Konig, and K.H. Wagner, *Exercise-induced DNA damage: is there a relationship with inflammatory responses?* Exerc Immunol Rev, 2008. **14**: p. 51-72.
86. Miller, S.L., C.M. Maresh, L.E. Armstrong, C.B. Ebbeling, S. Lennon, and N.R. Rodriguez, *Metabolic response to provision of mixed protein-carbohydrate supplementation during endurance exercise*. Int J Sport Nutr Exerc Metab, 2002. **12**(4): p. 384-97.
87. Miller, S.L., P.C. Gaine, C.M. Maresh, L.E. Armstrong, C.B. Ebbeling, L.S. Lamont, and N.R. Rodriguez, *The effects of nutritional supplementation throughout an endurance run on leucine kinetics during recovery*. Int J Sport Nutr Exerc Metab, 2007. **17**(5): p. 456-67.
88. Lunn, W.R., S.M. Pasiakos, M.R. Colletto, K.E. Karfonta, J.W. Carbone, J.M. Anderson, and N.R. Rodriguez, *Chocolate Milk & Endurance Exercise Recovery: Protein Balance, Glycogen & Performance*. Med Sci Sports Exerc, 2011.
89. Habouzit, E., H. Richard, H. Sanchez, N. Koulmann, B. Serrurier, R. Monnet, R. Ventura-Clapier, and X. Bigard, *Decreased muscle ACE activity enhances functional response to endurance training in rats, without change in muscle oxidative capacity or contractile phenotype*. J Appl Physiol, 2009. **107**(1): p. 346-53.
90. Woods, D.R., S.E. Humphries, and H.E. Montgomery, *The ACE I/D polymorphism and human physical performance*. Trends Endocrinol Metab, 2000. **11**(10): p. 416-20.
91. Gayagay, G., B. Yu, B. Hambly, T. Boston, A. Hahn, D.S. Celermajer, and R.J. Trent, *Elite endurance athletes and the ACE I allele--the role of genes in athletic performance*. Hum Genet, 1998. **103**(1): p. 48-50.
92. Karp, J.R., J.D. Johnston, S. Tecklenburg, T.D. Mickleborough, A.D. Fly, and J.M. Stager, *Chocolate milk as a post-exercise recovery aid*. Int J Sport Nutr Exerc Metab, 2006. **16**(1): p. 78-91.
93. Lee, J.K., R.J. Maughan, S.M. Shirreffs, and P. Watson, *Effects of milk ingestion on prolonged exercise capacity in young, healthy men*. Nutrition, 2008. **24**(4): p. 340-7.
94. Cockburn, E., P. Robson-Ansley, P.R. Hayes, and E. Stevenson, *Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage*. Eur J Appl Physiol, 2012.

95. Cockburn, E., E. Stevenson, P.R. Hayes, P. Robson-Ansley, and G. Howatson, *Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage*. Appl Physiol Nutr Metab, 2010. **35**(3): p. 270-7.
96. Assumpcao Cde, O., L.C. Lima, F.B. Oliveira, C.C. Greco, and B.S. Denadai, *Exercise-induced muscle damage and running economy in humans*. ScientificWorldJournal, 2013. **2013**: p. 189149.
97. Josse, A.R. and S.M. Phillips, *Impact of milk consumption and resistance training on body composition of female athletes*. Med Sport Sci, 2012. **59**: p. 94-103.
98. Thomas, D.T., L. Wideman, and C.A. Lovelady, *Effects of calcium and resistance exercise on body composition in overweight premenopausal women*. J Am Coll Nutr, 2010. **29**(6): p. 604-11.
99. Thomas, D.T., L. Wideman, and C.A. Lovelady, *Effects of a dairy supplement and resistance training on lean mass and insulin-like growth factor in women*. Int J Sport Nutr Exerc Metab, 2011. **21**(3): p. 181-8.
100. Josse, A.R., J.E. Tang, M.A. Tarnopolsky, and S.M. Phillips, *Body composition and strength changes in women with milk and resistance exercise*. Med Sci Sports Exerc, 2010. **42**(6): p. 1122-30.
101. Roy, B.D., K. Luttmer, M.J. Bosman, and M.A. Tarnopolsky, *The influence of post-exercise macronutrient intake on energy balance and protein metabolism in active females participating in endurance training*. Int J Sport Nutr Exerc Metab, 2002. **12**(2): p. 172-88.
102. Millard-Stafford, M., W.L. Childers, S.A. Conger, A.J. Kampfer, and J.A. Rahnert, *Recovery nutrition: timing and composition after endurance exercise*. Curr Sports Med Rep, 2008. **7**(4): p. 193-201.
103. Burke, L.M., J.A. Winter, D. Cameron-Smith, M. Enslen, M. Farnfield, and J. Decombaz, *Effect of Intake of Different Dietary Protein Sources on Plasma Amino Acid Profiles at Rest and After Exercise*. Int J Sport Nutr Exerc Metab, 2012.
104. Tresaco, B., G. Bueno, I. Pineda, L.A. Moreno, J.M. Garagorri, and M. Bueno, *Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children*. J Physiol Biochem, 2005. **61**(2): p. 381-8.
105. *Food Sources of Vitamin D*. 2013 [cited 2013 19 June]; Available from: <http://www.dietitians.ca/Nutrition-Resources-A-Z/Factsheets/Vitamins/Food-Sources-of-Vitamin-D.aspx>.
106. Vieth, R., P.C. Chan, and G.D. MacFarlane, *Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level*. Am J Clin Nutr, 2001. **73**(2): p. 288-94.
107. Ikekawa, N. and N. Koizumi, *Separation of vitamin D metabolites and their analogues by high-pressure liquid chromatography*. J Chromatogr, 1976. **119**: p. 227-32.
108. Tanaka, Y., H.F. DeLuca, and N. Ikekawa, *High-pressure liquid chromatography of vitamin D metabolites and analogs*. Methods Enzymol, 1980. **67**: p. 370-85.
109. *Vitamin D blood levels of Canadians*. 2013 [cited 2013 13/08]; Available from: <http://www.statcan.gc.ca/pub/82-624-x/2013001/article/11727-eng.htm>.

110. Hulver, M.W., D. Zheng, C.J. Tanner, J.A. Houmard, W.E. Kraus, C.A. Slentz, M.K. Sinha, W.J. Pories, K.G. MacDonald, and G.L. Dohm, *Adiponectin is not altered with exercise training despite enhanced insulin action*. Am J Physiol Endocrinol Metab, 2002. **283**(4): p. E861-5.
111. Stuart, C.A., M.A. South, M.L. Lee, M.P. McCurry, M.E. Howell, M.W. Ramsey, and M.H. Stone, *Insulin Responsiveness in Metabolic Syndrome after Eight Weeks of Cycle Training*. Med Sci Sports Exerc, 2013.
112. Kerksick, C.M., C.J. Rasmussen, S.L. Lancaster, B. Magu, P. Smith, C. Melton, M. Greenwood, A.L. Almada, C.P. Earnest, and R.B. Kreider, *The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training*. J Strength Cond Res, 2006. **20**(3): p. 643-53.
113. Van Berendoncks, A.M., A. Garnier, P. Beckers, V.Y. Hoymans, N. Possemiers, D. Fortin, V. Van Hoof, S. Dewilde, C.J. Vrints, R. Ventura-Clapier, and V.M. Conraads, *Exercise training reverses adiponectin resistance in skeletal muscle of patients with chronic heart failure*. Heart, 2011. **97**(17): p. 1403-9.
114. Niu, K., Y. Kobayashi, L. Guan, H. Monma, H. Guo, Y. Cui, A. Otomo, M. Chujo, and R. Nagatomi, *Low-fat dairy, but not whole-/high-fat dairy, consumption is related with higher serum adiponectin levels in apparently healthy adults*. Eur J Nutr, 2013. **52**(2): p. 771-8.
115. Oh, S., K. Tanaka, E. Warabi, and J. Shoda, *Exercise Reduces Inflammation and Oxidative Stress in Obesity-Related Liver Diseases*. Med Sci Sports Exerc, 2013.
116. Kriketos, A.D., S.K. Gan, A.M. Poynten, S.M. Furler, D.J. Chisholm, and L.V. Campbell, *Exercise increases adiponectin levels and insulin sensitivity in humans*. Diabetes Care, 2004. **27**(2): p. 629-30.
117. Tong, X., J. Dong, Z. Wu, W. Li, and L. Qin, *[Whey protein improves insulin resistance via the increase of antioxidant capacity in model rats]*. Wei Sheng Yan Jiu, 2011. **40**(5): p. 617-9.
118. Hoffman, J.R., and Falvo, M.J., *Protien - which is best?* Journal of Sports Science and Medicine, 2004. **3**: p. 118-130.
119. Struijk, E.A., A. Heraclides, D.R. Witte, S.S. Soedamah-Muthu, J.M. Geleijnse, U. Toft, and C.J. Lau, *Dairy product intake in relation to glucose regulation indices and risk of type 2 diabetes*. Nutr Metab Cardiovasc Dis, 2012.
120. Canada, H. *Physical Activity*. 2011 [cited 2013 12 August]; Available from: <http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/activit-eng.php>.

## 12. Appendix

### 12.1 Pre- and post-testing data

#### 12.1.1 Nutritional intake

Table A1. Total energy and macronutrient intake by group and time point

| Dietary Intake<br>per day | DEI           |               | DEA           |               | CEI           |               | CEA           |               |
|---------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                           | Pre           | Post          | Pre           | Post          | Pre           | Post          | Pre           | Post          |
| Kcal                      | 2845 ±<br>424 | 1931 ±<br>337 | 2215 ±<br>297 | 2622 ±<br>245 | 2373 ±<br>225 | 2148 ±<br>531 | 2641 ±<br>395 | 2165 ±<br>153 |
| CHO (Kcal)                | 1500 ±<br>224 | 945 ±<br>171  | 878 ±<br>181  | 1354 ±<br>110 | 1089 ±<br>138 | 1162 ±<br>233 | 1029 ±<br>104 | 1028 ±<br>164 |
| CHO (g)                   | 310 ± 56      | 236 ± 43      | 220 ± 45      | 339 ± 28      | 272 ± 35      | 291 ± 58      | 257 ± 26      | 257 ± 41      |
| PRO (Kcal)                | 423 ± 75      | 303 ± 51      | 357 ± 57      | 407 ± 75      | 518 ±<br>105  | 418 ± 51      | 457 ± 39      | 384 ± 46      |
| PRO (g)                   | 106 ± 19      | 76 ± 13       | 89 ± 14       | 102 ± 19      | 129 ± 26      | 105 ± 13      | 114 ± 10      | 96 ± 11       |
| FAT (Kcal)                | 830 ±<br>102  | 688 ±<br>132  | 820 ±<br>212  | 900 ±<br>147  | 857 ± 92      | 858 ±<br>153  | 1170 ±<br>356 | 722 ± 17      |
| FAT (g)                   | 92 ± 11       | 76 ± 15       | 91 ± 24       | 100 ± 16      | 95 ± 10       | 95 ± 17       | 130 ± 40      | 80 ± 2        |

Abbreviations: DEI – Dairy-Exercise-Immediate group; DEA – Dairy-Exercise-Alternate group; CEI – Carbohydrate-Exercise-Immediate group; CEA – Carbohydrate-Exercise-Alternate group; Kcal: kilocalorie; CHO: carbohydrate intake; PRO: protein intake; FAT: fat intake; g - grams. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. No significant differences between groups or time-points.

### 12.1.2 Serum vitamin D

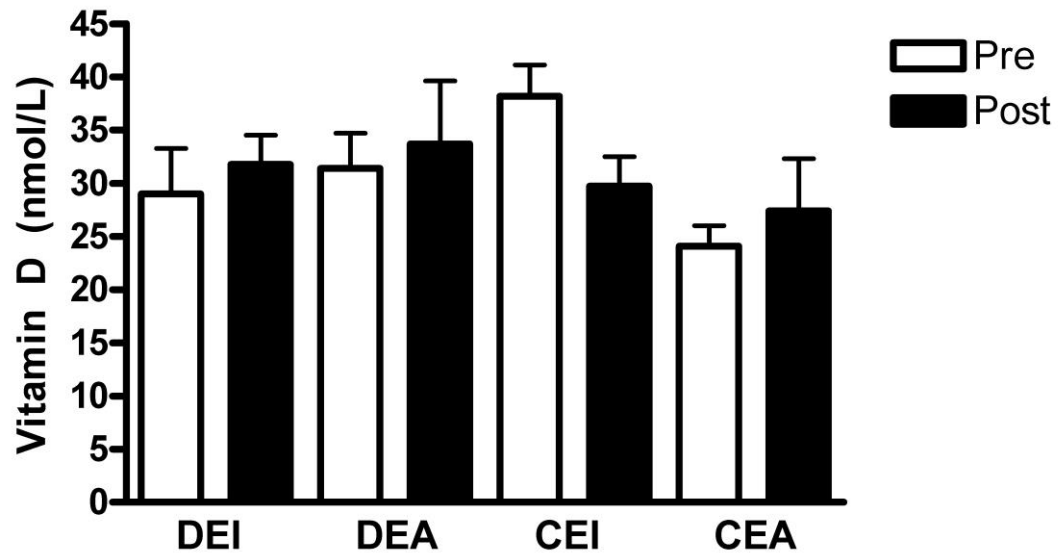


Figure A1. Vitamin D status by group and time point.

Abbreviations: DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. No significant differences between groups or time points.

### 12.1.3 Body composition

Table A2. Body composition measures by group and time point.

|                | DEI    |         | DEA    |         | CEI    |         | CEA    |         |
|----------------|--------|---------|--------|---------|--------|---------|--------|---------|
|                | Pre    | Post    | Pre    | Post    | Pre    | Post    | Pre    | Post    |
| Body mass (kg) | 80 ± 4 | 80 ± 3  | 83 ± 6 | 84 ± 5  | 91 ± 6 | 91 ± 6  | 86 ± 4 | 87 ± 3  |
| Fat mass (kg)  | 20 ± 2 | 21 ± 1  | 23 ± 4 | 23 ± 4  | 28 ± 3 | 27 ± 1  | 25 ± 2 | 26 ± 2  |
| Lean mass (kg) | 56 ± 2 | 57 ± 2* | 57 ± 1 | 58 ± 1* | 63 ± 4 | 64 ± 4* | 59 ± 3 | 60 ± 4* |

Abbreviations: DEI – Dairy-Exercise-Immediate group; DEA – Dairy-Exercise-Alternate group; CEI – Carbohydrate-Exercise-Immediate group; CEA – Carbohydrate-Exercise-Alternate group; kg: kilogram. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. \*Increase of lean mass across all groups from baseline ( $p < 0.05$ ).



### 12.1.4 $VO_2$ peak

Table A3.  $VO_2$ peak measures by group and time point.

| $VO_2$ peak                   | DEI        |                        | DEA        |                        | CEI        |                        | CEA        |                        |
|-------------------------------|------------|------------------------|------------|------------------------|------------|------------------------|------------|------------------------|
|                               | Pre        | Post                   | Pre        | Post                   | Pre        | Post                   | Pre        | Post                   |
| Absolute (L/min)              | 3.7 ± 0.2  | 4.0 ± 0.1 <sup>^</sup> | 3.3 ± 0.2  | 3.2 ± 0.3 <sup>^</sup> | 3.9 ± 0.4  | 4.3 ± 0.4 <sup>^</sup> | 3.1 ± 0.4  | 3.3 ± 0.4 <sup>^</sup> |
| Relative (ml/kg/min)          | 46.9 ± 1.2 | 51.8 ± 3.2*            | 40.5 ± 5.0 | 41.9 ± 5.3*            | 43.8 ± 3.1 | 47.7 ± 3.0*            | 35.3 ± 4.0 | 37.6 ± 3.3*            |
| Relative to LM (ml/kg LM/min) | 66.7 ± 2.9 | 66.9 ± 1.0             | 57.1 ± 5.0 | 57.5 ± 5.9             | 64.0 ± 2.8 | 69.4 ± 2.9             | 52.3 ± 5.7 | 55.1 ± 5.7             |

Abbreviations: DEI – Dairy-Exercise-Immediate group; DEA – Dairy-Exercise-Alternate group; CEI – Carbohydrate-Exercise-Immediate group; CEA – Carbohydrate-Exercise-Alternate group; L/min: litres per minute; ml/kg/min: millilitres per kilogram per minute; LM: lean mass; ml/kg LM/min: millilitres per kilogram of lean mass per minute. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. <sup>^</sup>Trend toward significance for absolute  $VO_2$ peak to be higher compared to baseline ( $p < 0.07$ ). \*Increase in relative  $VO_2$ peak values from baseline ( $p < 0.05$ ).

### 12.1.5 Serum lipids

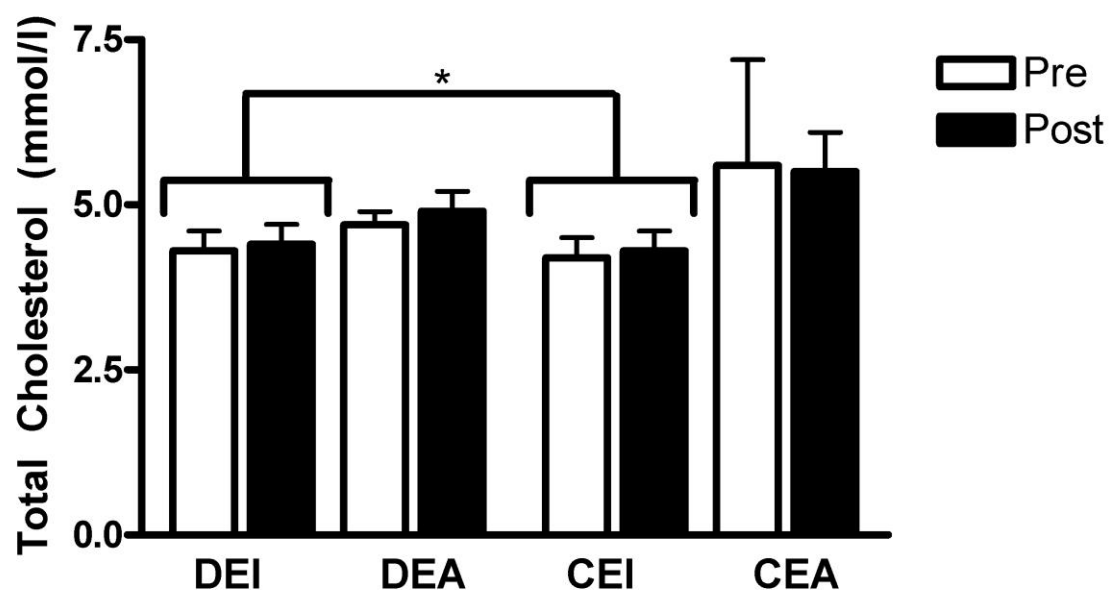


Figure A2. Total cholesterol by group and time point.

Abbreviations: DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. \*TCHOL was significantly lower overall for DEI and CEI groups in comparison to DEI and DEA groups ( $p < 0.05$ ); this effect is not attributable to the intervention.

Table A4. Serum lipids by group and time point

| Marker<br>(mmol/L) | DEI        |            | DEA       |           | CEI        |            | CEA       |           |
|--------------------|------------|------------|-----------|-----------|------------|------------|-----------|-----------|
|                    | Pre        | Post       | Pre       | Post      | Pre        | Post       | Pre       | Post      |
| TCHOL              | 4.3 ± 0.3* | 4.3 ± 0.3* | 4.7 ± 0.3 | 4.7 ± 0.2 | 4.2 ± 0.3* | 4.3 ± 0.3* | 5.6 ± 0.6 | 5.5 ± 0.6 |
| LDL                | 2.6 ± 0.3* | 2.6 ± 0.2* | 2.9 ± 0.3 | 2.9 ± 0.3 | 2.5 ± 0.2* | 2.6 ± 0.3* | 3.5 ± 0.5 | 3.6 ± 0.6 |
| TGs                | 1.8 ± 0.4  | 1.5 ± 0.2  | 1.1 ± 0.2 | 0.9 ± 0.1 | 1.1 ± 0.2  | 1.0 ± 0.1  | 2.0 ± 0.4 | 2.4 ± 0.9 |
| HDL                | 1.1 ± 0.1  | 1.1 ± 0.1  | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.1  | 1.2 ± 0.1  | 1.2 ± 0.1 | 1.2 ± 0.1 |

Abbreviations: TCHOL: Total cholesterol; LDL: Low-density lipoprotein cholesterol; TGs: Triglycerides; HDL: High-density lipoprotein cholesterol; DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. \*TCHOL and LDL values were significantly lower overall for DEI and CEI groups in comparison to DEA and CEA groups ( $p < 0.05$ ); this finding is not attributable to the intervention. No other significant effects noted.

### 12.1.6 Inflammatory and anti-inflammatory markers

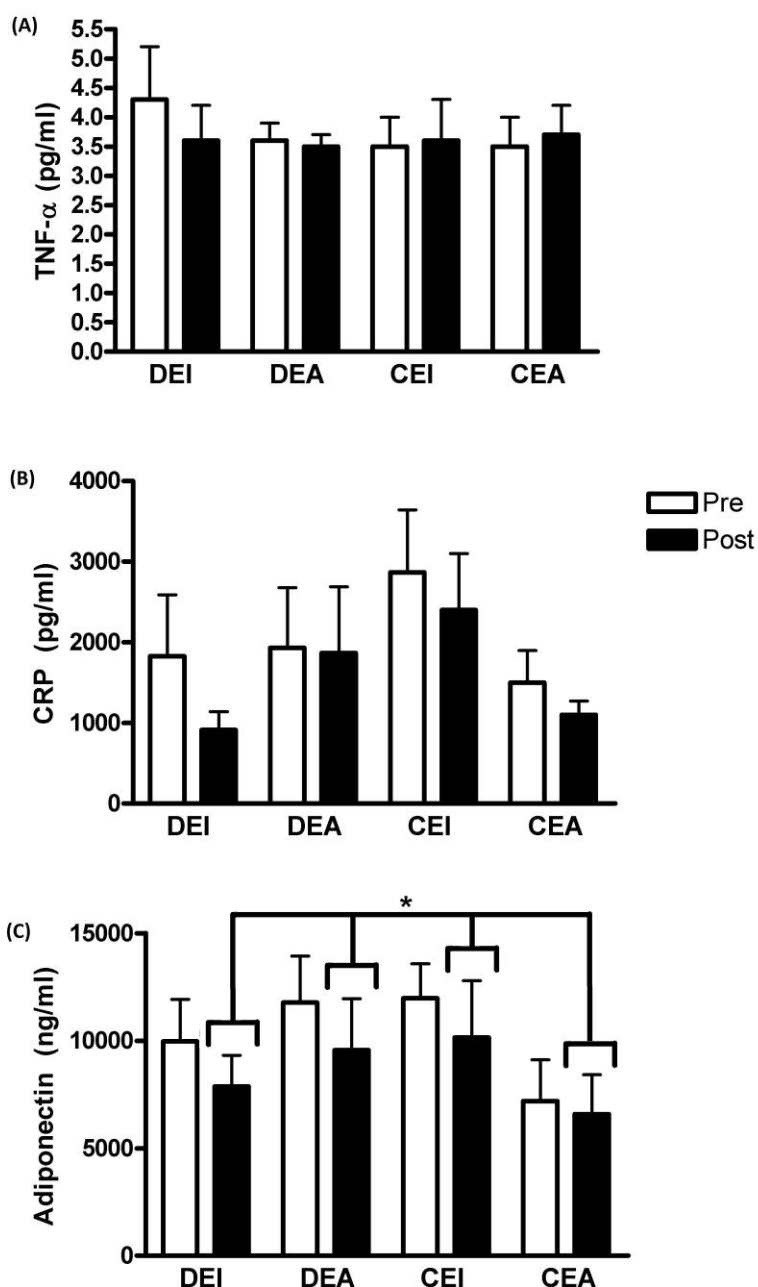


Figure A3. TNF- $\alpha$  (A), CRP (B) and adiponectin (C) by group and time point. Abbreviations: TNF- $\alpha$  – Tumour necrosis factor alpha; CRP – C-reactive protein; DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. \*All groups showed a decrease in adiponectin levels compared to baseline (p < 0.05).

Table A5. Fasting inflammatory markers by group and time point

| Marker                       | DEI               |                | DEA               |                | CEI               |                    | CEA           |                |
|------------------------------|-------------------|----------------|-------------------|----------------|-------------------|--------------------|---------------|----------------|
|                              | Pre               | Post           | Pre               | Post           | Pre               | Post               | Pre           | Post           |
| TNF $\alpha$<br>(pg/ml)      | 4.3 $\pm$ 0.9     | 3.6 $\pm$ 0.6  | 3.6 $\pm$ 0.3     | 3.5 $\pm$ 0.2  | 3.5 $\pm$ 0.5     | 3.6 $\pm$ 0.7      | 3.5 $\pm$ 0.5 | 3.7 $\pm$ 0.5  |
| CRP<br>(ng/ml)               | 1.8 $\pm$ 0.8     | 0.9 $\pm$ 0.2  | 1.9 $\pm$ 0.7     | 1.9 $\pm$ 0.8  | 2.9 $\pm$ 0.8     | 2.4 $\pm$ 0.7      | 1.5 $\pm$ 0.4 | 1.1 $\pm$ 0.2  |
| Adiponectin<br>( $\mu$ g/ml) | 10.0 $\pm$<br>2.0 | 7.9 $\pm$ 1.4* | 11.8 $\pm$<br>2.1 | 9.6 $\pm$ 2.4* | 12.0 $\pm$<br>1.6 | 10.1 $\pm$<br>2.7* | 7.2 $\pm$ 1.9 | 6.6 $\pm$ 1.8* |

Abbreviations: TNF- $\alpha$  – Tumour necrosis factor alpha; pg/ml – picograms per millilitre; CRP – C-reactive protein; ng/ml – nanograms per millilitre;  $\mu$ g/ml – micrograms per millilitre; DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. \*All groups showed a decrease in adiponectin levels compared to baseline (p < 0.05).

### 12.1.7 Metabolic markers

Table A6. Fasting serum metabolic markers by group and time point

| Marker           | DEI       |           | DEA       |           | CEI       |           | CEA       |           |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                  | Pre       | Post      | Pre       | Post      | Pre       | Post      | Pre       | Post      |
| Glucose (mmol/l) | 3.1 ± 0.1 | 3.2 ± 0.1 | 3.2 ± 0.1 | 3.2 ± 0.1 | 3.2 ± 0.1 | 3.2 ± 0.1 | 3.7 ± 0.4 | 3.3 ± 0.1 |
| Leptin (ng/ml)   | 6.1 ± 1.8 | 6.6 ± 2.2 | 8.4 ± 3.0 | 6.9 ± 2.2 | 8.8 ± 1.8 | 6.9 ± 1.9 | 6.9 ± 2.3 | 8.0 ± 2.3 |
| Glucagon (pg/ml) | 49 ± 8    | 50 ± 8    | 77 ± 15   | 53 ± 12   | 57 ± 20   | 57 ± 9    | 52 ± 10   | 47 ± 10   |
| Insulin (pg/ml)  | 529 ± 96  | 549 ± 112 | 522 ± 137 | 385 ± 79  | 424 ± 114 | 329 ± 64  | 446 ± 66  | 556 ± 143 |
| HOMA index *     | 2.1 ± 0.4 | 2.3 ± 0.5 | 2.0 ± 0.5 | 1.7 ± 0.3 | 1.7 ± 0.5 | 1.4 ± 0.3 | 2.1 ± 0.3 | 1.4 ± 0.3 |

Abbreviations: HOMA – Homeostatic Model Assessment; DEI – Dairy-Exercise-Immediate; DEA – Dairy-Exercise-Alternate; CEI – Carbohydrate-Exercise-Immediate; CEA – Carbohydrate-Exercise-Alternate. DEI: n = 7. DEA: n = 5. CEI: n = 6. CEA: n = 4. No significant differences between groups or time points. \*Trend toward significance for HOMA index to be lower post-intervention ( $p < 0.08$ ).